

Detection of Antimicrobial Resistance Genes in *Alcaligenes faecalis* Isolated from Urine Samples of HIV-Positive Individuals

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Abstract: Urinary tract infections (UTIs) pose a significant health challenge for individuals living with HIV, often involving opportunistic pathogens with escalating antimicrobial resistance. This study focused on *Alcaligenes faecalis* isolates recovered from urine samples of HIV-positive individuals attending a selected health facility in Akure, Nigeria. The study employed 16S rRNA gene sequencing for identification and PCR-based screening for key resistance genes. Three isolates (SM1, SM5, and SM11) were confirmed as *A. faecalis*, exhibiting a consistent genotypic profile with presence of *qnrB* (fluoroquinolone resistance) at 400 bp and *aadA* (aminoglycoside resistance) at 300 bp, while negative for *blaOXA-114*, *AcrB*, *BlaGes*, *sul3*, and *FosA*. These findings highlight a core resistome in these environmental-origin opportunists, underscoring the need for genotypic surveillance in immunocompromised populations to guide empirical therapy. The absence of *blaOXA-114* further validates species distinction from related *Achromobacter* species.

Key word: *Alcaligenes faecalis*, Antimicrobial resistance genes, HIV, Urinary tract infection, PCR

INTRODUCTION

Individuals with Human Immune deficiency Virus (HIV) face heightened susceptibility to urinary tract infections due to impaired immune responses and frequent exposure to healthcare settings, where opportunistic pathogens thrive (Gebremedhin *et al.*, 2024). In fact, *Escherichia coli* remains the predominant etiologic agent, non-enteric Gram-negative bacilli, including *Alcaligenes faecalis*, are increasingly implicated in complicated urinary tract infections (UTIs) among this group, particularly in resource-limited environments (Adonu *et al.*, 2025). *Alcaligenes faecalis*, a motile, oxidase-positive rod typically found in soil and water, emerges as an opportunistic invader in the urinary tract, exploiting urinary stasis or catheterization common in HIV management.

The clinical burden is compounded by the organism's intrinsic and acquired resistance mechanisms, rendering standard regimens like fluoroquinolones and aminoglycosides ineffective (Chen *et al.*, 2020). Recent reports from Ethiopia documented *A. faecalis* among rare UTI isolates in HIV patients, with universal resistance to amoxicillin and frequent multidrug patterns, including ciprofloxacin and gentamicin (Gebremedhin *et al.*, 2024). Similarly, a Nigerian cohort identified multidrug-

resistant *A. faecalis* as a novel UTI pathogen, showing 75% resistance across tested agents except select carbapenems, emphasizing its therapeutic recalcitrance (Adonu *et al.*, 2025). These phenotypic observations align with global trends of extensively drug-resistant (XDR) *A. faecalis* strains, where efflux pumps and plasmid-mediated elements drive broad-spectrum resistance (Chen *et al.*, 2020).

Despite these advances, critical knowledge gaps persist in the molecular frameworks of resistance in *A. faecalis* from HIV-associated UTIs. Most studies rely on phenotypic susceptibility testing, overlooking genotypic determinants like plasmid-borne *qnr* genes or integron-associated *aadA*, which facilitate rapid dissemination (Jacoby *et al.*, 2014). This disconnect hampers hypothesis-driven insights into resistance dynamics, such as whether environmental *A. faecalis* strains in HIV cohorts harbor a conserved resistome shaped by anthropogenic pressures like antibiotic overuse in antiretroviral therapy settings.

This study hypothesized that *A. faecalis* isolates from HIV-positive urine samples would carry a defined set of antimicrobial resistance genes (ARGs), including those for fluoroquinolones and aminoglycosides, contributing to their opportunistic virulence. The primary aim was to confirm species

identity via 16S rRNA sequencing and screen for clinically relevant ARGs using validated PCR protocols.

MATERIALS AND METHODS

Bacterial Isolates, Culture Conditions, and Genomic DNA Extraction: Single colonies of each *A. faecalis* isolate from the urine samples were inoculated into 1.5 ml of standard liquid growth medium and incubated for 48 hours at 28°C on an orbital shaker (Eppendorf, Germany/ New Brunswick Scientific, Innova® 2100 Platform Shaker, M1194-0002; 15 to 500 RPM). Genomic DNA was extracted using a modified CTAB-based protocol (Trindade *et al.*, 2007). Cells were pelleted by centrifugation at $4600 \times g$ for 5 mins, resuspended in 520 μ l TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), and lysed with 15 μ l 20% SDS and 3 μ l proteinase K (20 mg/ml at 37°C for 1 hour. Following addition of 100 μ l 5 M NaCl and 80 μ l 10% CTAB (in 0.7 M NaCl), the mixture was incubated at 65°C for 10 minutes and chilled on ice for 15 minutes. Phases were separated with chloroform:isoamyl alcohol (24:1 v/v) by centrifugation at $7200 \times g$ for 20 mins. Deoxyribose nucleic acid (DNA) was precipitated with 0.6 volumes cold isopropanol overnight at -20°C, pelleted at $13000 \times g$ for 10 minutes, washed with 70% ethanol, air-dried, and resuspended in 50 μ l TE buffer for storage at -20°C.

DNA Quantification and Quality Assessment: The DNA concentration and purity were evaluated using a NanoDrop 2000 spectrophotometer, targeting $A_{260}/A_{280} \approx 1.8$ and $A_{260}/A_{230} > 2.0$. Samples were normalized to 50 ng/ μ l with nuclease-free water.

16S rRNA Gene Amplification, Purification, and Sequencing for Species Identification: A near full-length 16S rRNA fragment (~1500 bp) was amplified using primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGATCCAGCC-3') (Frank *et al.*, 2008). Reactions (50 μ l) included 10 μ l 5 $\times$ GoTaq buffer, 3 μ l 25

mM MgCl₂, 1 μ l 10 mM dNTPs, 1 μ l each primer (10 pmol/ μ l), 0.3 U Taq polymerase, 8 μ l template DNA, and water. Cycling: 94°C/5 min; 30 cycles of 94°C/30 s, 50°C/60 s, 72°C/90 s; 72°C/10 min. Products were verified by 1.5% agarose gel electrophoresis, purified via ethanol precipitation (40 μ l product + 7.6 μ l 3 M sodium acetate + 240 μ l 95% ethanol, -20°C/30 min, centrifuged at $13000 \times g$ /10 min at 4°C, washed with 70% ethanol, dried, resuspended in 20 μ l water, and sequenced using BigDye Terminator v3.1 on an ABI 3130xl Genetic Analyzer.

Bioinformatic Analysis and Species Identification: Sequences were trimmed, assembled in BioEdit, and queried against NCBI nr/nt via BLASTn, assigning identity at >99% identity, >99% coverage, E-value 0.0. MEGA 6 was used for alignments if needed.

PCR-Based Screening for Antimicrobial Resistance Genes: Isolates were screened for seven ARGs using singleplex PCR with primers as shown in Table 1. Reactions (25 μ l) used standard master mix, 1 μ l each primer (10 pmol), ~100 ng DNA. Cycling: 94°C/5 min; 35 cycles of 94°C/30 s, 49–53°C/40 s, 72°C/60–90 s; 72°C/10 min. Amplicons were resolved on 1.5% agarose gels (1 $\times$ TAE, 0.5 μ g/ml ethidium bromide) at 110–120 V for 45–60 min, visualized under UV with a 100 bp ladder.

Ethical approval: Ethical approval was obtained from the State Health Research Ethics Committee (SHREC) of Ondo State, Nigeria (OSHREC 23/9/2025/385). Patients consent were sought before the collection of samples.

RESULTS

The 16S rRNA sequencing identified three isolates as *A. faecalis* (SM1, SM5, SM11), with >99.6% identity, 99–100% coverage, and E-value 0.0, as shown in Table 2, while Figure 1 shows their amplicon band size of ~1500 bp. The phylogenetic and evolutionary relationship of the isolates among other opportunistic pathogens is shown in Figure 2. The SM1, SM5 and

SM11 clustered with known *A. faecalis* strain OM661183.1, revealing close genetic relatedness.

All three isolates harbored *qnrB* (~400 bp, slightly larger than expected 389 bp) and

aadA (300 bp) genes as shown in Figure 3. No amplicons were detected for *blaOXA-114* (944 bp), *AcrB*, *BlaGes*, *sul3*, and *FosA* genes. The genotypic profile across the isolates is presented in Table 3.

Table 1: Oligonucleotide Primers Used for the Detection of Antimicrobial Resistance Genes in *Alcaligenes faecalis* from Urine Samples of HIV-Positive Individuals

Target Gene	Primer Sequence (5' → 3')	Expected Amplicon Size (bp)	References
<i>blaOXA-114</i>	F: ACGCCTGAACCCTTTTATCC R: ATCGACAGGCCGCGCAGT	944	Turton <i>et al.</i> (2011)
<i>AcrB</i>	F: FPGAAAGGCCAACAGCTTAAC R: GAGCTGGAGTCAGGATCAAC	800	Liu <i>et al.</i> (2013)
<i>BlaGes</i>	F: GTTTTGCAATGTGCTCAACG R: TGCCATAGCAATAGGCGTAG	387	Haghighi and Reza (2022)
<i>aadA</i>	F: GCACGACGACATCATTCCG R: ACCAAATGCGGGACAACG	300	Drum (2003)
<i>qnrB</i>	F: CCTGAGCGGCACTGAATTTAT R: GTTTGCTGCTCGCCAGTCGA	389	Wu <i>et al.</i> (2008)
<i>sul3</i>	F: TCCGTTTACGCGAATTGGTGCAG R: TTCGTTTACGCGCTTACACCAGC	126	Lavilla <i>et al.</i> (2014)
<i>FosA</i>	F: GTCCACTCACAACGGTTTGC R: CGACATACCAAGGGAAATCC	311	Goh <i>et al.</i> (2006)

Table 2: Molecular Identity of *A. faecalis* Isolates from Urine Samples of HIV-Positive Individuals

Sample ID	Scientific Name	Max Score	Total Score	E value	Percentage Identity (%)	Accession
1 (SM1)	<i>Alcaligenes faecalis</i>	1790	1790	0	100.00	PV762942
5 (SM5)	<i>Alcaligenes faecalis</i>	1764	1764	0	99.69	PV762945
11 (SM11)	<i>Alcaligenes faecalis</i>	1770	1770	0	99.79	PV762946

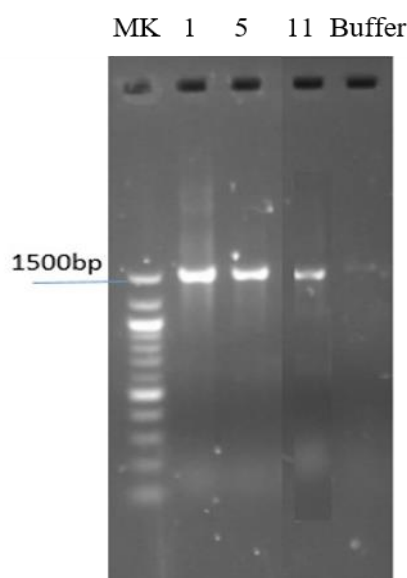


Figure 1: Electrophoretic Gel Image of the Purified DNA Bands (~1500 bp) of *A. faecalis* Isolates. MK – Molecular Weight Ladder, 1, 5 and 11 are SM1, SM5, and SM11 Identities of *A. faecalis* Isolates Respectively

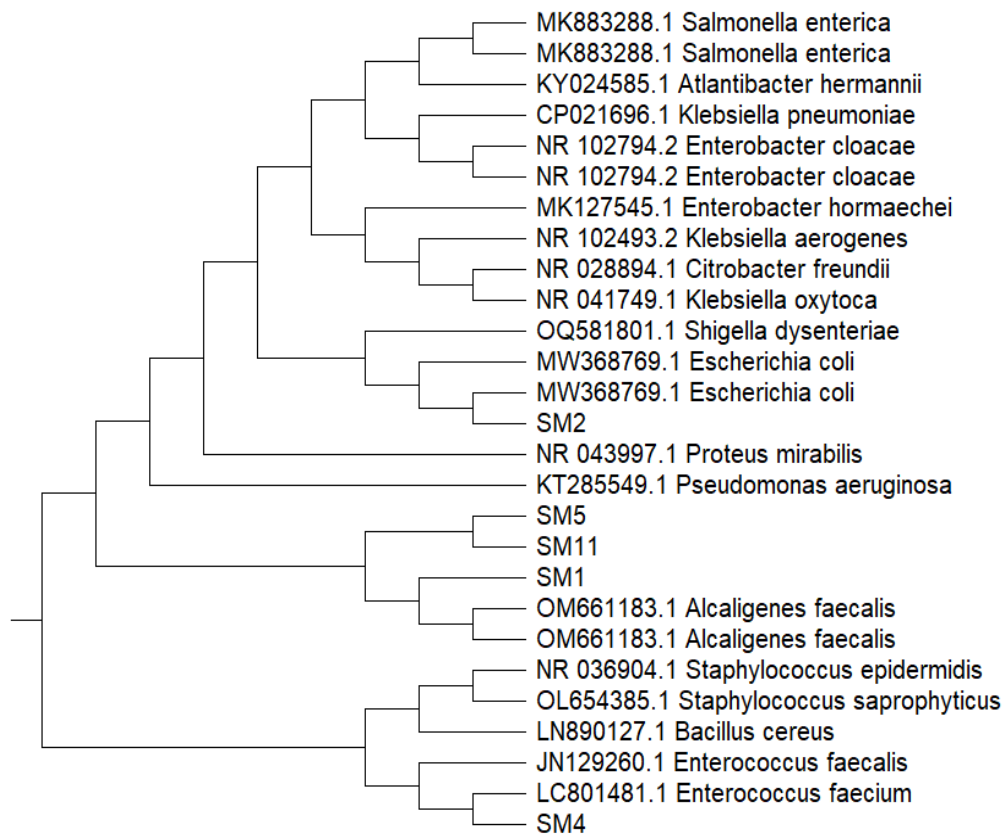


Figure 2: Phylogenetic Profile of *Alcaligenes faecalis* Strains Isolated from Urine Samples of HIV-Positive Individuals

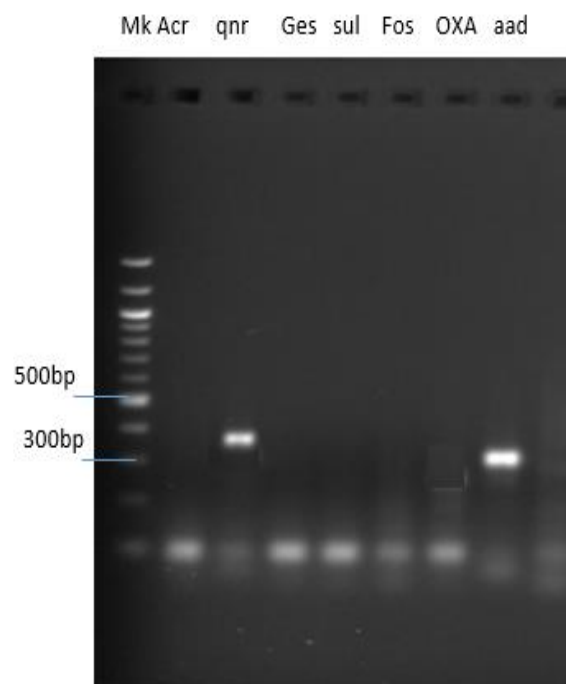


Figure 3: Amplified PCR Products of Fluoroquinolone Resistance Gene (*qnrB*) at ~500 bp and Aminoglycoside Resistance Gene (*aadA*) at 300 bp in *A. faecalis* Isolates. MK – Molecular Weight Ladder

Table 3: Genotypic Profile of *A. faecalis* Isolates from Urine Samples of HIV-Positive Individuals

Target Genes	<i>A. faecalis</i> SM1	<i>A. faecalis</i> SM5	<i>A. faecalis</i> SM11
<i>bla-OXA-114</i>	-	-	-
<i>AcrB</i>	-	-	-
<i>BlaGes</i>	-	-	-
<i>aadA</i>	+	+	+
<i>qnrB</i>	+	+	+
<i>sul3</i>	-	-	-
<i>FosA</i>	-	-	-

Keys: + = Positive – Present, - = Negative – Absent

DISCUSSION

The isolation and molecular confirmation of *Alcaligenes faecalis* from the urine samples of HIV-positive individuals corroborates their increasing recognition as opportunistic pathogens in immunocompromised individuals. This is consistent with recent epidemiological reviews that highlight an elevated risk of complicated UTIs and colonization by atypical, often resistant, bacteria in people living with HIV, even in the era of effective antiretroviral therapy (Koukou *et al.*, 2021). The identification of these bacterial isolates among the subjects aligns with the opportunistic niche, where immunosuppression facilitates colonization by environmental microbes (Gebremedhin *et al.*, 2024). The 16S rRNA data, bolstered by negative *bla-OXA-114* screening, firmly distinguishes these isolates from *Achromobacter xylosoxidans*, a common confound in non-fermenter identification (Turton *et al.*, 2011).

This methodological rigor addresses a noted pitfall in prior phenotypic studies, where misidentification may inflate resistance reports (Chen *et al.*, 2020). The uniform presence of *qnrB* and *aadA* suggests a conserved resistome, likely acquired via integrons or plasmids prevalent in aquatic reservoirs (Drum, 2003; Wu *et al.*, 2008). The *qnrB* role in low-level fluoroquinolone resistance mirrors patterns in Ethiopian HIV-UTI isolates, where *A. faecalis* showed >60% ciprofloxacin non-susceptibility, potentially driven by such PMQR elements (Gebremedhin *et al.*, 2024). Similarly, *aadA*-mediated aminoglycoside resistance echoes

the 75% gentamicin resistance in Nigerian cases, highlighting selective pressures from empirical UTI therapy in HIV care (Adonu *et al.*, 2025). The amplicon size discrepancy for *qnrB* (~500 bp) may indicate allelic variants or insertion elements, warranting sequencing for mobility assessment.

Interestingly, absence of *AcrB*, despite its commonality in Gram-negatives, implies reliance on other efflux or enzymatic mechanisms, consistent with XDR profiles lacking upregulated pumps (Liu *et al.*, 2013; Chen *et al.*, 2020). Negative results for *BlaGes*, *sul3*, and *FosA* further delineate a streamlined profile, contrasting broader ARG arrays in hospital *A. faecalis* (Goh *et al.*, 2006; Lavilla *et al.*, 2014; Haghighi and Reza, 2022). This baseline informs hypothesis testing on environmental against clinical evolution, filling gaps in HIV-specific genotypic data.

CONCLUSION

This study confirms the presence of *Alcaligenes faecalis* as an opportunistic pathogen in urine samples from HIV patients. The 16S rRNA sequencing and targeted PCR screening revealing a uniform genotypic resistome characterized by *qnrB* (fluoroquinolone resistance) and *aadA* (aminoglycoside resistance) across all three isolates. The absence of *blaOXA-114* reinforces species specificity, distinguishing these strains from related *Achromobacter* species. These findings underscore the environmental origin and conserved resistance mechanisms of *A. faecalis*, posing a therapeutic challenge in

immunocompromised hosts and highlighting the value of molecular surveillance for

guiding empirical antibiotic selection in HIV-associated UTIs.

REFERENCES

- Adonu, C., Onyi, P. N., Nwobodo, H. A., Omeje, K. O., and Eze, C. C. (2025). First report of urinary tract infections with multidrug resistant *Alcaligenes faecalis* and *Staphylococcus cohnii* in Enugu State, Nigeria. *Journal of Medical Laboratory and Diagnosis*, 17(1):1–8.
- Chen, Y.-C., Hsueh, P.-R., and Liao, C.-H. (2020). Extensively drug-resistant *Alcaligenes faecalis* infection. *BMC Infectious Diseases*, 20(1), Article 833.
- Drum, D. J. (2003). Integron-associated aadA detection]. *Antimicrobial Agents and Chemotherapy*, 47(5):1653–1658.
- Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Luscombe, C. A., and Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology*, 74(8):2461–2470.
- Gebremedhin, K. B., Yisma, E., Alemayehu, H., Medhin, G., Belay, G., Bopegamage, S., Amogne, W., and Egualé, T. (2024). Urinary tract infection among people living with human immunodeficiency virus attending selected hospitals in Addis Ababa and Adama, central Ethiopia. *Frontiers in Public Health*, 12, Article 1394842.
- Goh, E. B., Yim, G., Tsui, W., McClure, J., Surette, M. G., and Davies, J. (2006). Identification of a putative operon involved in fructooligosaccharide utilization by *Bacteroides thetaiotaomicron*. *Journal of Bacteriology*, 188(1):441–453.
- Haghighi, S., and Goli, H. R. (2022). High prevalence of *bla*VEB, *bla*GES and *bla*PERgenes in beta-lactam resistant clinical isolates of *Pseudomonas aeruginosa*. *AIMS Microbiology*, 8(2):193–204.
- Jacoby, G. A., Strahilevitz, J. and Hooper, D. C. (2014). Plasmid-mediated quinolone resistance. *Microbiology Spectrum* 2(5): PLAS-0006-2013.
- Koukou, D., Kourouma, S., and Coffie, P. A. (2021). Bacterial infections in people living with HIV in Africa in the combination antiretroviral therapy era: A review. *The Lancet HIV*, 8(12): e781-e792.
- Lavilla L., L., Benomar, N., Pandal, A. I., Olatz, M., Calero, P. and Abriouel, H. (2014). Diversity, distribution and quantification of antibiotic resistance genes in goat and lamb slaughterhouse surfaces and meat products. *PLoS ONE*, 9(11):Article e114252.
- Liu, P. K., and Yen, M. Y. (2013). AcrB primers in efflux detection. *Journal of Microbiology, Immunology and Infection*, 46(4):285–291.
- Trindade, F., Ambrosini, A., Costa, P. B., and Passaglia, L. M. P. (2007). A modified CTAB protocol for DNA extraction from soybean and meat samples. *Genetics and Molecular Biology*, 30(3):438–442.
- Turton, J. F., Mustafa, N., Shah, J., Hampton, C. V., Pike, R., and Kenna, D. T. (2011). Identification of *Achromobacter xylosoxidans* by detection of the blaOXA-114-like gene intrinsic in this species. *Diagnostic Microbiology and Infectious Disease*, 70(4):408–411.
- Wu, J.-J., Ko, W.-C., Wu, H.-M., and Yan, J.-J. (2008). Prevalence of Qnr determinants amongst bloodstream isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *Journal of Antimicrobial Chemotherapy*, 61(6):1234–1235.