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Molecular Characterization of Bacterial Isolates from Selected Maternity Centres in Rivers State, Nigeria

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ABSTRACT

Environmental isolates from maternity centres require reliable identification before their significance for maternal and neonatal care can be assessed. This study examined the molecular characteristics of ten selected bacterial isolates recovered during monthly sampling at six maternity centres in Rivers State, Nigeria, from January to December 2025. Each of three senatorial districts contributed a clinic and a traditional birth attendant facility. Sampling covered water, air, delivery mats, delivery beds, kidney trays and attendants' palms. DNA extraction with a ZR Fungal/Bacterial DNA Miniprep kit preceded 16S ribosomal RNA (rRNA) gene amplification with primers 27F and 1525R. Sanger sequencing used BigDye Terminator v3.1 chemistry and an Applied Biosystems 3500 Genetic Analyzer, with genetic analysis in Geneious Prime. DNA was visible in ten extraction lanes, and amplification products occurred near 1,500 base pairs. Pairwise identities to the closest reported database references ranged from 69.46% to 98.72%. The highest value, 98.72%, occurred for TA2 S1 against Klebsiella pneumoniae strain CCFM8370; SBR 5 had 81.55% identity to a different K. pneumoniae reference. Identities to the three Escherichia coli references were 96.61%, 91.68% and 92.57%. Comparisons with Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Staphylococcus aureus and Staphylococcus epidermidis references yielded 77.91%, 82.36%, 75.52%, 76.61% and 69.46%, respectively. Six of the ten identities were below 90%. The molecular results therefore varied substantially in their support for the named reference organisms and did not uniformly confirm species identity. These environmental sequence comparisons provide no evidence of transmission to mothers or newborns. Low-identity isolates require confirmatory characterization, while routine environmental monitoring, hand hygiene and decontamination of delivery-contact items remain relevant to infection prevention.

Keywords: *16S rRNA gene; Sanger sequencing; bacterial identification; maternity centres; sequence similarity; environmental isolates.*

1. Introduction

Equipment and surfaces used in maternity and neonatal care can harbour bacteria, but recovery from the care environment does not establish infection in a patient. Investigations of neonatal intensive-care equipment have recovered *Escherichia coli*, *Klebsiella* species and *Staphylococcus aureus* [1]. Bacterial contamination has also been documented on medical equipment and in the indoor air of neonatal and paediatric wards [2]. Identifying such isolates helps distinguish organisms associated with environmental or skin sources from those that warrant further investigation for their potential clinical relevance.

Contamination is not always apparent from visual inspection. An investigation of maternity units and labour rooms in Gujarat, India, detected bacterial contamination at sites considered visually clean [3]. A Kenyan hospital study likewise recovered clinically relevant bacteria from frequently touched surfaces in several departments, including maternity and newborn units [4].

These observations justify examining cultured isolates more closely where routine tests leave their identity uncertain. Differences in local conditions and sampling methods nevertheless limit direct comparison between facilities.

The 16S rRNA gene combines relatively conserved regions, which permit amplification across bacterial groups, with variable regions that help distinguish taxa. Sequencing can therefore supplement phenotypic tests when biochemical reactions overlap or differ from expected patterns [5]. Identification still depends on the quality and length of the sequence, the reference collection and the separation between the best match and competing matches [6]. The species name attached to a search result must be assessed against that evidence rather than accepted solely because it appears first.

Reference curation and the region sequenced influence the taxonomic detail that can be reported. A curated 16S reference collection supported broad bacterial classification, although genus- and species-level assignments were not equally reliable [7].

Evaluation of full-length and partial sequences showed greater discriminatory information across the full gene than within individual short variable regions [8]. Relationships between 16S similarity and genome-wide relatedness provide useful taxonomic context, but similarity thresholds must be interpreted alongside sequence quality, alignment coverage and the bacterial group concerned [9], [10].

A separate question is whether an environmental organism has reached a patient. Genome-resolved comparisons of neonatal rooms and infants have identified strains shared between the two settings [11]. Combined environmental and newborn sampling has also examined microbial overlap during neonatal admission [12]. Environmental 16S comparisons alone do not provide equivalent evidence. In the present study, molecular characterization addressed the identity of recovered isolates, not the attribution of maternal or neonatal infection to a sampled source.

This study investigated selected bacterial isolates recovered during twelve months of environmental sampling at maternity centres in Rivers State, Nigeria. It examined DNA extraction, 16S rRNA amplification and the closest database sequence matches. The objective was to characterize this selected isolate collection and assess the taxonomic information supported by its molecular results.

2. Materials and Methods

2.1. Study setting and isolate collection

The investigation involved six maternity centres across Rivers East/Central, Rivers South East and Rivers West senatorial districts. Each district contributed one clinic and one traditional birth attendant (TBA) facility. The centres were Rumukwurusi Model Primary Health Centre and a TBA home in Rumukwurusi; Oyigbo Model Primary Health Centre and a TBA home in Oyigbo; and Saint Peter's Clinic, Bonny, and a TBA home in Bonny. The observational study used repeated monthly environmental sampling from January to December 2025.

The sampling programme covered water, air, delivery mats, delivery beds, kidney trays and attendants' palms. One sample of each type was collected per centre per month, yielding 432 samples over the year. Water was collected aseptically in sterile bottles. Nutrient agar plates were exposed for ten minutes at approximately one metre above ground for passive air sampling. Delivery-contact items and palms were sampled with sterile swabs, which were returned to physiological saline and transported in a cooler at 2-8°C. Water and swab suspensions underwent tenfold serial dilution and spread plating on nutrient and MacConkey agar, followed by incubation at 37°C for 24-48 hours. Pure cultures of ten selected isolates were used for molecular analysis. These isolates constituted a subset of the environmental collection. Electrophoretic observations comprised ten DNA-extract lanes and ten amplification-product lanes. Permission to conduct the study was obtained from the Ministry of Health and Primary Health Care Management Board.

2.2. DNA extraction and electrophoresis

DNA was extracted using the ZR Fungal/Bacterial DNA Miniprep kit (Zymo Research). A 2 ml bacterial broth sample was transferred to a ZR BashingBead lysis tube and mixed with 750 µl lysis solution. Mechanical disruption was performed at maximum speed for more than 5 minutes, followed by centrifugation at greater than 10,000 × g for 1 minute. Up to 400 µl supernatant was passed through a Zymo-Spin IV filter at 7,000 × g for 1 minute.

The filtrate was mixed with 1,200 µl Fungal/Bacterial DNA Binding Buffer and loaded onto a Zymo-Spin IIC column in 800 µl portions, with centrifugation at 10,000 × g for 1 minute after each loading step.

The column was washed with 200 µl DNA Pre-Wash Buffer and then 500 µl Fungal/Bacterial DNA Wash Buffer, with centrifugation at 10,000 × g for 1 minute after each wash. DNA was eluted into a clean 1.5 ml tube using 100 µl DNA Elution Buffer and centrifugation at 10,000 × g for 30 seconds.

Extracted DNA and PCR products were examined on 1% and 2% agarose gels, respectively. Gels were prepared in 1× Tris-acetate-EDTA buffer with 10 µl EZ Vision DNA stain per 100 ml gel preparation. Loading buffer was added to samples before electrophoresis. Gels were run at 80-150 V for approximately 1-1.5 hours, and bands were visualized using a UV transilluminator.

2.3. Amplification of the 16S rRNA gene

Polymerase chain reaction (PCR) targeted the 16S rRNA gene using the primers in Table 1. The use of conserved primer-binding regions for bacterial 16S rRNA amplification underlies sequence-based phylogenetic characterization [13]. Each 25 µl reaction contained 12.5 µl Taq 2X Master Mix (New England Biolabs), 1 µl each of 10 µM forward and reverse primers, 2 µl DNA template and 8.5 µl nuclease-free water.

Initial denaturation was performed at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 45 seconds. A final extension at 72°C for 7 minutes was followed by a hold at 10°C. Amplification products were examined against a 1 kb DNA ladder, with the target product identified at approximately 1,500 base pairs (bp).

Table 1: Primers used for bacterial 16S rRNA gene amplification

Primer	Direction	Sequence, 5' to 3'
27F	Forward	AGAGTTTGATCMTGGCTCAG
1525R	Reverse	AAGGAGGTGWTCCARCCGC

Key: M = A or C; W = A or T; R = A or G; bp = base pairs. Primer positions do not establish the length of the final usable sequence read.

2.4. Sequencing and database comparison

Amplified fragments were sequenced using BigDye Terminator v3.1 cycle-sequencing chemistry on an Applied Biosystems 3500 Genetic Analyzer, following the manufacturer's instructions. The method uses chain-terminating nucleotides to determine nucleotide sequence [14]. Genetic analyses were performed in Geneious Prime.

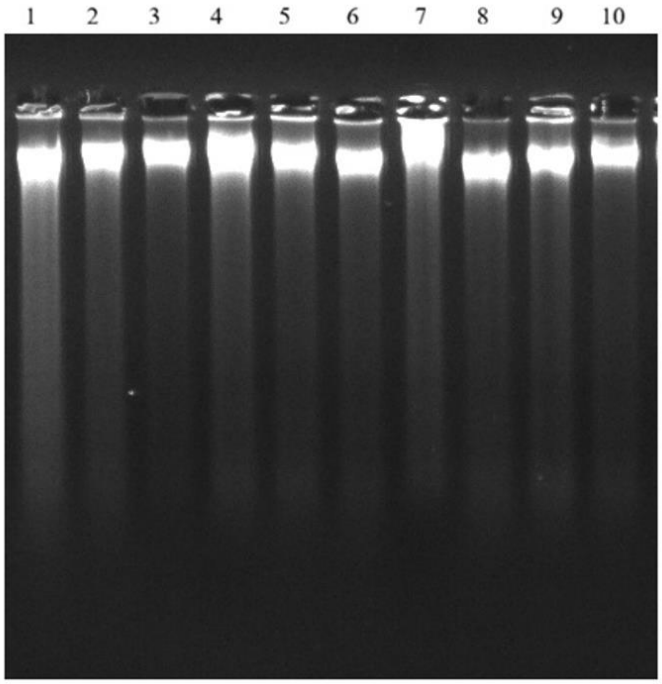
Sequence comparisons were summarized by isolate code, accession of the related reference organism, closest-match descriptor and percentage pairwise identity. Local-alignment methods identify sequence similarities rather than organism pathogenicity [15], [16]. Reference strain names describe the related database sequences; they were not assigned as strain identities of the maternity-centre isolates.

2.5. Descriptive analysis

Individual sequence identities and their range were summarized descriptively, without treating them as prevalence estimates for the maternity centres. No facility-type, seasonal or clinical-outcome tests were applied to the ten selected sequence records. Similarity values were interpreted with reference to the limitations of 16S-based identification, particularly when matches were distant from the named reference [17].

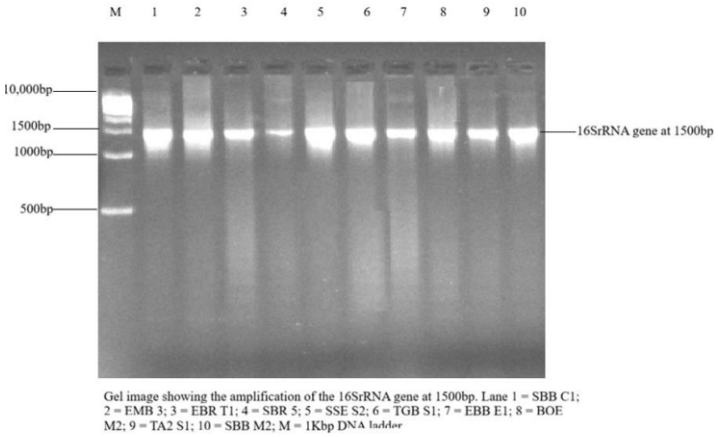
3. Results

DNA-containing material was visible in all ten numbered extraction lanes, with differences in band intensity and downward smearing (Fig. 1). The amplification gel showed products near the marked 1,500 bp position in lanes 1-10, alongside the molecular-size ladder (Fig. 2). The extraction and amplification images therefore documented successive stages of molecular processing. Band presence did not provide a measurement of sequence identity or the length of the final sequence alignment.



Gel image showing high molecular weight DNA extracted from the isolates. Lane 1 = SBB C1; 2 = EMB 3; 3 = EBR T1; 4 = SBR 5; 5 = SSE S2; 6 = TGB S1; 7 = EBB E1; 8 = BOE M2; 9 = TA2 S1; 10 = SBB M2

Figure 1: Electrophoretic appearance of DNA extracted from the bacterial isolates.
Key: Lanes 1-10 = SBB C1, EMB 3, EBR T1, SBR 5, SSE S2, TGB S1, EBB E1, BOE M2, TA2 S1 and SBB M2, respectively.



Gel image showing the amplification of the 16SrRNA gene at 1500bp. Lane 1 = SBB C1; 2 = EMB 3; 3 = EBR T1; 4 = SBR 5; 5 = SSE S2; 6 = TGB S1; 7 = EBB E1; 8 = BOE M2; 9 = TA2 S1; 10 = SBB M2; M = 1Kbp DNA ladder

Figure 2: Amplification products of the bacterial 16S rRNA gene at approximately 1,500 bp.
Key: M = 1 kb DNA ladder; lanes 1-10 = SBB C1, EMB 3, EBR T1, SBR 5, SSE S2, TGB S1, EBB E1, BOE M2, TA2 S1 and SBB M2, respectively; bp = base pairs. The size marker refers to amplification products, not independently measured sequence-read lengths.

The ten isolates included three *Escherichia coli*-labelled matches, two *Klebsiella*-labelled matches, two *Staphylococcus*-labelled matches and one match each to *Pseudomonas*, *Proteus* and *Serratia* references (Table 2). Identities ranged from 69.46% to 98.72%. TA2 S1 showed the highest identity, matching *Klebsiella pneumoniae* strain CCFM8370 at 98.72%, while SBR 5 matched *K. pneumoniae* strain BA1559 at 81.55%. BOE M2, EBR T1 and EMB 3 matched *E. coli* references at 96.61%, 91.68% and 92.57%, respectively. EBB E1 matched *Pseudomonas aeruginosa* strain PO44UC at 77.91%, and SBB C1 matched *Proteus mirabilis* strain BPAS2 at 82.36%. The remaining values were 75.52% for the *Serratia marcescens* reference, 76.61% for the *Staphylococcus aureus* reference and 69.46% for the *Staphylococcus epidermidis* reference. Six comparisons were below 90% identity.

The isolates included water-associated and delivery-contact organisms. BOE M2 and EMB 3 were water isolates from the Bonny and Rumukwurusi TBA centres, respectively. Kidney trays accounted for EBB E1 at Rumukwurusi Model Primary Health Centre, SBB M2 at TBA Oyigbo and SSE S2 at TBA Bonny. SBB C1 was associated with air at Rumukwurusi Model Primary Health Centre, TA2 S1 with an attendant's palm at TBA Rumukwurusi, and TGB S1 with a delivery bed at Saint Peter's Clinic, Bonny.

Table 2: Closest database reference matches for the selected bacterial isolates

Isolate code	Related reference accession	Closest reference descriptor	Pairwise identity (%)
BOE M2	MT192515	*Escherichia coli* strain N14-2	96.61
EBB E1	OR461701	*Pseudomonas aeruginosa* strain PO44UC	77.91
EBR T1	PQ220169	*Escherichia coli* strain 10	91.68
EMB 3	MT921164	*Escherichia coli* strain TEM 129	92.57
SBB C1	KX156178	*Proteus mirabilis* strain BPAS2	82.36
SBB M2	PZ213219	*Serratia marcescens* strain UIS0526	75.52
SBR 5	CP036187.1	*Klebsiella pneumoniae* strain BA1559	81.55
SSE S2	LC575195	*Staphylococcus aureus* IRQBAS88	76.61
TA2 S1	KJ803927	*Klebsiella pneumoniae* strain CCFM8370	98.72
TGB S1	MH997761.1	*Staphylococcus epidermidis* strain 29	69.46

Key: *Accessions identify the related database references, not deposited sequences of the study isolates. Reference organism and strain names are closest-match descriptors, not confirmed reference-strain identities. Percentage identity is not query coverage.*

4. Discussion

The principal molecular finding was the wide variation in similarity between the selected isolates and their closest named references. TA2 S1 had the highest identity among the ten comparisons, while six comparisons were below 90%. This distinction matters more than the number of species names returned by the search. Evaluation of a curated reference collection [7] demonstrated that the reliability of bacterial sequence classification depends on both the reference collection and the classification approach. The present records should therefore be understood as a set of closest sequence matches with unequal taxonomic confidence, not a uniformly confirmed list of seven bacterial species.

Amplification at approximately 1,500 bp is consistent with targeting a near-full-length 16S rRNA gene, but amplicon size does not establish the amount of sequence that entered a database alignment. Full-length sequencing can provide greater taxonomic discrimination than analysis of individual short variable regions [8]. The region sequenced and the analytical workflow can also alter taxonomic assignments, particularly at species level [18]. These studies help explain why the visible amplification products and the low identities address different analytical questions: a product of the expected size is evidence of amplification, whereas identification depends on the sequence subsequently obtained from it.

Recent isolate-identification research reinforces this distinction. A clinical diagnostic study [19] compared long-read 16S sequencing with a shorter Sanger approach for 153 clinical isolates and found greater genus-level resolution with the longer-read platform. Their study included whole-genome assessment of discrepancies. The present investigation did not compare sequencing platforms or evaluate identification accuracy against a genome-based reference. Its ten records consequently cannot establish the sensitivity, specificity or species-level accuracy of the molecular workflow.

The two *K. pneumoniae*-labelled matches illustrate the difference between a close and a distant reference comparison. TA2 S1 had 98.72% identity, compared with 81.55% for SBR 5. A 98.65% 16S similarity threshold has been related to species demarcation through genome-wide comparisons [9]; that taxonomic relationship is not a guarantee that an individual database hit provides a definitive clinical identification. Whole-genome sequencing of clinical isolates has revealed misidentification among members of the *K. pneumoniae* species complex [20]. TA2 S1 is therefore best described as having a close *K. pneumoniae*-labelled reference match, without assigning it to reference strain CCFM8370. The lower identity of SBR 5 does not support confirmation as *K. pneumoniae* or assignment to strain BA1559.

The three *E. coli*-labelled matches had identities of 96.61%, 91.68% and 92.57%. These results do not justify assigning an enteropathogenic group, a virulence profile or an infection outcome. Even high-quality sequence-based identification encounters difficulty among closely related *Escherichia* and *Shigella* organisms [21]. The present identities provide still less support for fine taxonomic discrimination. Their value lies in documenting the observed comparisons and identifying isolates for confirmatory characterization, rather than establishing three definitively identified *E. coli* strains.

The *Staphylococcus*-labelled comparisons require similar restraint. Their identities of 76.61% and 69.46% are distant from the named *S. aureus* and *S. epidermidis* references and do not confirm those species.

Comparison of staphylococcal gene targets showed that discrimination among staphylococci could differ between 16S rRNA and alternative genes [22]. More generally, sequence identity carries information at different taxonomic ranks across bacterial and archaeal groups [10]. A low-identity hit should not be promoted automatically even to a firm genus assignment solely because the database returns a familiar species name.

The same issue applies to the *Pseudomonas*, *Proteus* and *Serratia* descriptors. Their identities of 77.91%, 82.36% and 75.52% are insufficient for confident identification of the named species. Work with bioaerosol-derived colonies [23] showed that colony purity, electropherogram quality and sequencing approach affected the interpretation of 16S results. Mixed templates are one possible explanation for ambiguous or low-quality sequences, but they were not demonstrated here. Other possibilities include poor base calling, limited usable sequence, inappropriate alignment or an unsuitable reference. The present data do not distinguish among these explanations.

Sequence quality and reference choice consequently remain central to interpretation. A diagnostic review [17] identified limitations arising from ambiguous sequences, database annotation and inadequate discrimination between close alternatives. A subsequent review [6] likewise stressed the importance of usable sequence length and match differentiation. Reference resources such as SILVA and EzBioCloud illustrate how sequence curation and taxonomic organization can support comparative analysis [24], [25]. Their relevance here is methodological: they were not additional databases used to generate the reported results.

Technical explanations for low identity should not be mistaken for demonstrated faults in these isolates. Chimeric 16S amplicons have been documented experimentally [26], and anomalies have been identified in public sequence records [27]. Reagent-derived DNA can also affect sequence-based microbial investigations, particularly at low biomass [28]. These studies establish reasons for using sequence and assay controls; they do not prove that any particular result in the present collection arose from contamination or chimera formation. Raw electropherograms, usable read lengths, query coverage and control results would be needed to investigate the cause of an individual low-identity match.

The recorded sample origins locate the molecular observations within particular maternity-care activities. The two water isolates, BOE M2 and EMB 3, had *E. coli*-labelled reference matches at the Bonny and Rumukwurusi TBA centres, respectively. Three kidney-tray isolates carried different reference descriptors at Rumukwurusi Model Primary Health Centre, TBA Oyigbo and TBA Bonny. These observations distinguish water-associated isolates from those recovered from reusable equipment; they do not establish a common contamination route. Hospital environmental investigations [1], [4] likewise recovered clinically relevant bacterial groups from care-environment surfaces and equipment, although differences in sampling and identification preclude direct comparison of frequencies. The air, palm and delivery-bed records extend the present molecular observations beyond water and trays. Sampling in neonatal and paediatric wards [2] also documented bacterial recovery from equipment and indoor air in neonatal and paediatric care. The overlap concerns the types of reference organisms encountered, not equivalent identification certainty. A selected ten-isolate collection cannot establish which organism predominated at any centre or in any sample matrix.

The distinction from patient-linked evidence is particularly important. A neonatal-unit investigation [11] used genome-resolved comparisons to investigate organisms shared between neonatal rooms and infants. A separate neonatal study [12] examined both environmental and newborn microbial communities over time. In contrast, no matched maternal or neonatal clinical isolates were analyzed in the present molecular comparison. The reference descriptors cannot therefore establish that a sampled surface, water source or attendant transmitted a bacterium to a patient. Genome-based taxonomic standards also require sequence-quality and relatedness evidence beyond a named reference match [29].

The practical implication is targeted environmental surveillance supported by reliable isolate identification. Cleaning and reprocessing of delivery beds, mats and kidney trays, together with hand hygiene, remain relevant irrespective of whether a low-identity isolate ultimately receives the initially reported species name. Maternity-unit findings [3] support microbiological assessment beyond visual cleanliness. Intervention studies in neonatal and delivery wards further show the importance of sustained environmental-hygiene measures rather than assumptions based on appearance alone [30], [31].

The molecular findings are limited by the small selected collection and by the absence of sequence-level quality metrics sufficient to validate every named match. DNA and amplification gels describe laboratory processing, not species-identification accuracy. The ten sequence records do not support seasonal, facility-type or population-prevalence inference, and the reference accessions do not constitute deposition of the study sequences. Confirmatory sequencing and comparison against suitable reference strains would strengthen identification. Genome-based methods would be required for any subsequent investigation of strain relatedness or transmission.

5. Conclusions

The ten selected maternity-centre isolates yielded bacterial DNA and 16S rRNA amplification products, with closest-reference identities ranging from 69.46% to 98.72%. TA2 S1 had the highest identity, at 98.72%, to a *K. pneumoniae*-labelled reference, whereas the second *K. pneumoniae*-labelled match, SBR 5, had 81.55% identity. Six comparisons were below 90%, limiting species-level interpretation of the named matches. These findings support molecular follow-up of cultured environmental isolates, with sequence-quality assessment and confirmatory identification before species-specific exposure claims are made. Environmental surveillance, hand hygiene and effective decontamination of delivery-contact items remain appropriate infection-prevention priorities.

Conflict of interest

The authors declare that there is no conflict of interest.

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