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ORIGINAL RESEARCH ARTICLE

Molecular Detection of Bla_{SHV} and Sul2 Genes in Multidrug-Resistant *Shigella flexneri* from Clinical Specimens in North-East Nigeria

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ABSTRACT
The continued detection and dissemination of multidrug-resistant (MDR) Shigella species pose a significant public health threat worldwide, especially in resource-limited regions such as the Northeast, Nigeria. Extended-Spectrum Beta-Lactamases (ESBLs) encoded by genes including Bla_{SHV} confer resistance to critical Beta-lactam antibiotics, and sulfonamide resistance genes such as sul2 complicate therapeutic regimens by mediating resistance to widely used drugs. The available data on ESBL and sulfonamide genes producing *Shigella* from African countries reflects limited technical resources, the absence of a national or regional surveillance system, and the lack of required publications for the limited data generated. This Study reports the genotypic detection confirming Bla_{SHV} and Sul2 resistance genes from multidrug-resistant *Shigella Flexneri* isolates from clinical specimens in Yobe State Specialist Hospital, Damaturu. The identified MDR phenotype *Shigella* species were collected, and their DNA was extracted and amplified using multiplex and simple PCR with various primer sets targeting specific sequences of the resistance genes. The gel images were then taken. Among five MDR *Shigella* strains tested by multiplex PCR against three β-lactamases (Bla_{TEM}, Bla_{CTX-M}, and Bla_{SHV}), Bla_{SHV} was detected in a *Shigella flexneri* strain. In this Study, no *blaTEM and blaCTX-M* genes were detected in any *of* the five products. In addition, two isolates (all *Shigella flexneri* strains) were found to harbor Sul2 genes, while only one MDR *Shigella flexneri* isolate harbored both Bla_{SHV} and Sul2 resistance genes, unlike the potential XDR *Shigella flexneri*, which carries only the Sul2 resistance gene. MDR *Shigella flexneri* strains isolated from Specialist Hospital, Damaturu, Yobe State, are a reservoir of disseminatable Bla_{SHV} and Sul2 resistance genes. However, despite its limitations, this Study highlights the urgent need for enhanced molecular antimicrobial stewardship and targeted interventions to curb the spread of resistant *Shigella* clones in this vulnerable region.

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INTRODUCTION
An estimated 165 million cases of shigellosis are reported annually, with about a million deaths worldwide. Most data from developing countries are culture-based based making it difficult to evaluate the accurate burden of *Shigella* infection in developing countries due to the limited scope of studies and lack of coordinated epidemiological surveillance systems (Somda *et al.,* 2025). New disease burden estimates from newly deployed molecular diagnostic assays with increased sensitivity suggest that *Shigella*-associated morbidity may be much greater than previous disease estimates from culture-based methods (Anderson *et al.,* 2023).

Extended-spectrum β-lactamases are mutant forms of broad-spectrum β-lactamases such as the TEM-1, TEM-2, and SHV-1 enzymes coded by genes located on transferable plasmids, which can easily spread from one organism to another (Shahnaij *et al.,* 2023). The global spread of ESBL-producing bacteria is largely driven by the dissemination of these three major gene families: Bla_{TEM}, Bla_{CTX-M}, and Bla_{SHV}, yet to know which genes encode ESBL in Nigeria, and to know the exact prevalence of every ESBL gene would be of importance (Hertz *et al.,* 2019). They encode enzymes capable of hydrolyzing and inactivating a wide range of β-lactam antibiotics. The accurate and rapid identification of these specific ESBL genotypes is of paramount importance for clinical diagnostics, epidemiological surveillance, and infection control (Losio *et al.,* 2015).

Just like the ESBL, the molecular detection of Sul2 genes is also crucial for understanding the epidemiology of sulfonamide resistance and for monitoring its prevalence in both clinical and agricultural settings (Titus *et al.,* 2023).

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Regional antimicrobial susceptibility profiles of each organism need to be monitored, as evidenced by the persistently shifting patterns of common species and the resistance of *Shigella* isolates in Nigeria (GLASS, 2025). Polymerase Chain Reaction (PCR) assays designed to target these genes provide a reliable means of identifying β-lactamase and sulfonamide-resistant bacteria, often with greater sensitivity and specificity than traditional susceptibility testing methods (Ososе *et al.,* 2025).

Phenotypically, multi-drug resistance prevalence has been reported in *Shigella* species from every state in the north-east and all other geopolitical zones of Nigeria, including: Damaturu (Saheed *et al.,* 2020), Maiduguri (Ngoshe *et al.,* 2017), Bauchi (Umar *et al.,* 2025), Adamawa (Tula *et al.,* 2022), all from the north-east, Nigeria. In the three zones of Odeda, south-west (Ajayi *et al.,* 2019), and Makudi, north-central (Richard *et al.,* 2023). A similar wide range of resistance phenotypes has been reported from Kano and Kaduna, northwest (Alhaji *et al.,* 2022; Lawal, 2017), and Enugu, southeast (Ososе *et al.,* 2025).

Genotypically, the components that are thought to be in charge of antimicrobial Resistance (AMR) acquisition and spread among organisms are clinically of immense importance. Whole-genome sequencing technology for routine microbiology has recently advanced, as widely reported (Leopold *et al.,* 2014) in developed countries. Yet, not many studies on the *Shigella* species' genetic makeup have been finished, despite substantial progress having been achieved in understanding the pathogenesis (Sahl *et al.,* 2015) and aid vaccine production (Anderson *et al.,* 2016; Mani *et al.,* 2016). The detection of blaSHV (beta-lactamase) and sul2 (sulfonamide resistance) genes by PCR is crucial for understanding multi-drug resistance (MDR) in Shigella flexneri. These genes are widely reported in clinical and foodborne isolates, contributing to resistance against beta-lactam and sulfonamide antibiotics.

On the other hand, Molecular studies is just getting attention in the sub-Sahara Africa (Baumgart *et al.,* 2024), no dedicated national or regional surveillance of diarrheagenic organisms and their AMR pattern in Nigeria (NAP, 2024) and the only available data are from institutional and academic research which are also minimal (Kahsay & Muthupandian, 2016; The *et al.,* 2016).

Currently, there is a published (widely indexed or accessible) literature gap regarding the PCR characterization of Shigella resistance genes in the northeastern, Nigeria (especially in Bauchi and Yobe States). This study aims to fill the grey research gap. The resistance genes detected are particularly important due to their plasmid-borne nature and high transfer potential, making them critical markers for surveillance in regions with a high AMR burden.

MATERIALS AND METHODS

Source of the *Shigella* Isolates

The phenotypic identification, susceptibility profile, MAR indices, Genotypic identification, and phylogenetic information of the five drug-resistant isolates used in this Study have been described in our previous Study (Saheed *et al.,* 2025).

DNA Extraction

DNA extraction was done using the kit method. After culturing isolates in lysogeny broth overnight, the isolates' total genomic DNA was extracted following the Accu prep Genomic DNA extraction kit (Bioneer) instructions.

Molecular Characterization of Multidrug-Resistant

PCR assay was used to amplify and detect drug resistance genes in *Shigella* isolates. The resistance genes (Bla_{SHV} and Sul2), which encode Extended Beta-Lactamases and Sulfonamides, were investigated in the tested antibiotics of the case study subjects. Multiplex PCR was carried out using optimized primers listed in Table 1. Multiplex PCR targeted ESBL genes (Bla_{CTX}, Bla_{TEM}, and Bla_{SHV}) with the following setup and conditions: 16 µL of distilled water (dH_2O), 1 µL each of forward and reverse primers (Bioneer), and 2 µL of template DNA. The reaction underwent a pre-denaturation at 95°C for 5 minutes, followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 40 seconds, repeated for 35 cycles. A final extension was performed at 72°C for 5 minutes using a Thermal Cycler PTC 100, MJ Research. The presence of sul2 was determined using the same PCR conditions, except for an annealing temperature of 50°C (Iliyasu *et al.,* 2018).

Agarose gel electrophoresis was performed using commercially available agarose (TAE). A 3 g sample of agarose was dissolved in a solution and heated in a microwave until fully dissolved. The solution was cooled in a water bath to 50-55°C. The gel casting tray was prepared by sealing the ends of the gel chamber with tape, and an appropriate number of combs were placed in the tray. 5 µL of ethidium bromide was added to the gel mixture, which was then cooled before being poured into the gel tray. The gel was allowed to solidify for 23 minutes at room temperature. Afterward, the combs were removed, the gel was placed in the electrophoresis chamber, and covered with buffer (TAE).

The DNA mixture and standards (Ladder) were loaded onto the gel, electrophoresed at 5V/cm for 1hour, and the DNA bands were visualized using a UV lightbox (gel imaging system, Biorad) (Abbasi *et al.,* 2019).

RESULTS

The results for the determination of Antibacterial drug resistance by the multiplex PCR technique are shown in Figure 1. Despite the wide range of phenotypic resistance to B-Lactamase antibiotics demonstrated by the *Shigella* isolates in this Study (Saheed *et al.,* 2025), no Bla_{CTX} gene or Bla_{TEM} gene was detected. Only Bla_{SHV} genes (824bp) were amplified from a *Shigella flexneri* strain . The amplification showed a broad visible band on the Bla_{SHV} lane. Other products (one strain of *Shigella flexneri* and three strains of *Shigella boydii*) in lanes 2, 3, 4, and 5 showed no detectable amplification of those particular β-lactamase genes. The negative control showed no bands, indicating

a clean PCR setup and no false positives. The positive control showed bands matching the expected positive control gene products, confirming the presence of bands for two positive controls (bla_{SHV} and bla_{CTX}).

Table 1: Oligonucleotide primers used for resistance gene amplification in this Study

	Primers	Sequences 5´-3´	Amplicon size(bp)	Reference
BlaTem	F –	GTA TCC GCT CAT GAG ACA ATA ACC	918	(Pan *et al.,* 2006)
	R –	CTG CCA ATG CTT AAT CAG TGA GGC ACC		
BlaCTX	F –	CGC TTT GCG ATG TGC AG	550	
	R –	ACC GCG ATA TCG TTG GT		
BlaSHV	F –	CGC CTG TGT ATT ATC TCC CTG TTA GCC	842	
	R –	TTG CCA GTG CTC GAT CAG CG		
Sul2	F –	AGGGGGCAGATGTGATCGAC	625	(De *et al.,* , 2020)
	R –	TGTGCGGATGAAGTCAGCTCC		

Table 2: Summary of the Multi-Drug-Resistant *Shigella* species Used in this Study

Shigella spp	MARI	Antibiotic Resistance Pattern	BlaSHV	Sul2
Shigella flexneri	0.412	CXM, CAZ, AMX, AMP, S, TET, CLN	+	+
Shigella boydii	0.176	CXM, CAZ, CLN	-	-
Shigella boydii	0.529	CXM, CAZ, CFM, CRO, AMX, AMP, S, CLN, SXT	-	-
Shigella boydii	0.529	CXM, CAZ, CFM, CRO, AMX, AMP, S, CLN, SXT	-	-
Shigella flexneri	0.882	CXM, CAZ, CFM, CRO, AMX, AMP, TZO, IP, CIP, LVX, S, AZM, TET, CLN, SXT	-	+

Keys: + = Detected, - = Not Detected, MARI = Multiple Antibiotics Resistance Index

The PCR product showed clear bands on lanes 1 and 5, detected at 625bp (Figure 2), which matches the positive control, indicating successful and specific amplification of the sul2 gene. The presence of a 625bp band is expected and a specific product for the sul2 gene amplification. Its appearance in the correct test lanes and the positive control confirms effective and specific PCR for the sul2 genes. Other products (all *Shigella boydii* strains) in lanes 2, 3, and 4 show no bands, similar to the negative control. This strongly suggests that the sul2 gene is not amplified in these samples. The presence of positive bands in other lanes and proper control results indicate a true negative (not detectable sul2 gene) in these PCR products.

DISCUSSION

Genotypically, two resistant genes were detected through PCR methods. Extended-spectrum β-lactamase resistance gene (BLA *SHV*) was detected in MDR *Shigella flexneri,* and the sulfonamide resistance gene (Sul2) was detected in two strains of MDR *Shigella flexneri,* including a potential XDR phenotype.

This study outcome has confirmed the presence of Bla_{SHV} in the PCR product with similar results reported at the same 800bp (Karmoker *et al.,* 2023; Tahmasbi *et al.,* 2024) using a single PCR. Using multiplex PCR, Shahnaij *et al.* (2023) reported amplified Bla_{SHV} at around 800bp when using standard primer sets similar to the ones used in this Study. Multiple studies confirm the presence of Bla_{SHV} in MDR *Shigella flexneri.* In one foodborne Study, all *Shigella* isolates, including *S. flexneri*, carried Bla_{SHV}, indicating its widespread role in beta-lactam resistance (Pakbin *et al.,* 2021). However, some regional studies (e.g., Iran) did not detect Bla_{SHV}, suggesting geographic variability (Sheikh *et al.,* 2020).

Meanwhile, Somda *et al.* (2025) compiled recent molecular studies worldwide reported that Bla_{SHV} is usually detected using PCR at the same base pair. Despite Dallal *et al.* (2018) not detecting Bla_{CTX-M}, $Bla_{TEM,}$ except Bla_{SHV} in *Shigella* species, others (Bajpai *et al.,* 2017; Kaur & Aggarwal, 2013; Michael & Saadi, 2018; Nkengkana *et al.,* 2023) have been able to detect all the genes (Bla_{CTX-M}, Bla_{TEM}, Bla_{SHV}) in some β-lactamase-resistant enterobacteria while only amplifying Bla_{SHV} in three (3) strains of *Klebsiella pneumoneae.* Bands for Bla_{SHV} were consistently observed at their expected amplicon size (usually 800–900bp depending on primers), and this protocol continues to inform contemporary research (Dallal *et al.,* 2018).

No verifiable Bla_{SHV} harboring *Shigella* species have been reported from Bauchi and Damaturu specifically, but multiplex PCR for detection of pathogenic *Shigella* species, different species-specific amplicons at 159 bp (*Shigella* genus), 248 bp (*S. boydii*), 503 bp (*S. sonnei*), and 314 bp (*S. flexneri*) have been reported in Terra, Iran (Ranjbar *et al.,* 2014) and India (Nandi *et al.,* 2023). This has, though, been reported in other bacteria such as *Salmonella typhi* (Umar *et al.,* 2025), *Escherichia coli* (Iliyasu *et al.,* 2018) from Bauchi, *Klebsiella pneumoneae* (Ngolo *et al.,* 2023) from Lafia, Nasarawa state, and other parts of the country (Mustapha *et al.,* 2023).

There are limited reports on the detection of sul2 in studies from Damaturu and Bauchi with 1.213 and 0.131 density compared to the positive control as found in this Study (1YS and 5YS) sulfonamide resistance sul1 gene has been reported from *Salmonella typhi* in Bauchi (Titus *et al.,* 2023) and (sul1 and sul2) well documented in Nigeria (Osose *et al.,* 2025).

In support of this Study, the sul2 gene, which confers resistance to sulfonamides, is frequently detected in clinical *S. flexneri* isolates, with 47% of strains from a pediatric cohort in China showing its presence. This presence was significantly associated with resistance to

sulfamethoxazole-trimethoprim (SXT) (Peng *et al.,* 2024). In Pakistan, sul2 was found in 63% of serotype 2b S. flexneri isolates, a serotype strongly linked to MDR (Nisa *et al.,* 2020).

In contrast, all SXT-resistant (sulfamethoxazole/trimethoprim) *Shigella* species isolated from different Chinese regions using PCR confirmed a high prevalence of sul genes targeted at 625bp, particularly sul1 and sul2, which commonly served as molecular markers for resistance surveillance (Isaiah *et al.,* 2025). This finding highlights the association of plasmid-borne sul genes at the expected 625bp region, correlating with phenotypic drug resistance (Asad *et al.,* 2025). Ranjbar *et al.* (2014) indicated that sul1, sul2, and sul3 genes are common in *Shigella* species worldwide, detected by PCR amplicons close to 625bp.

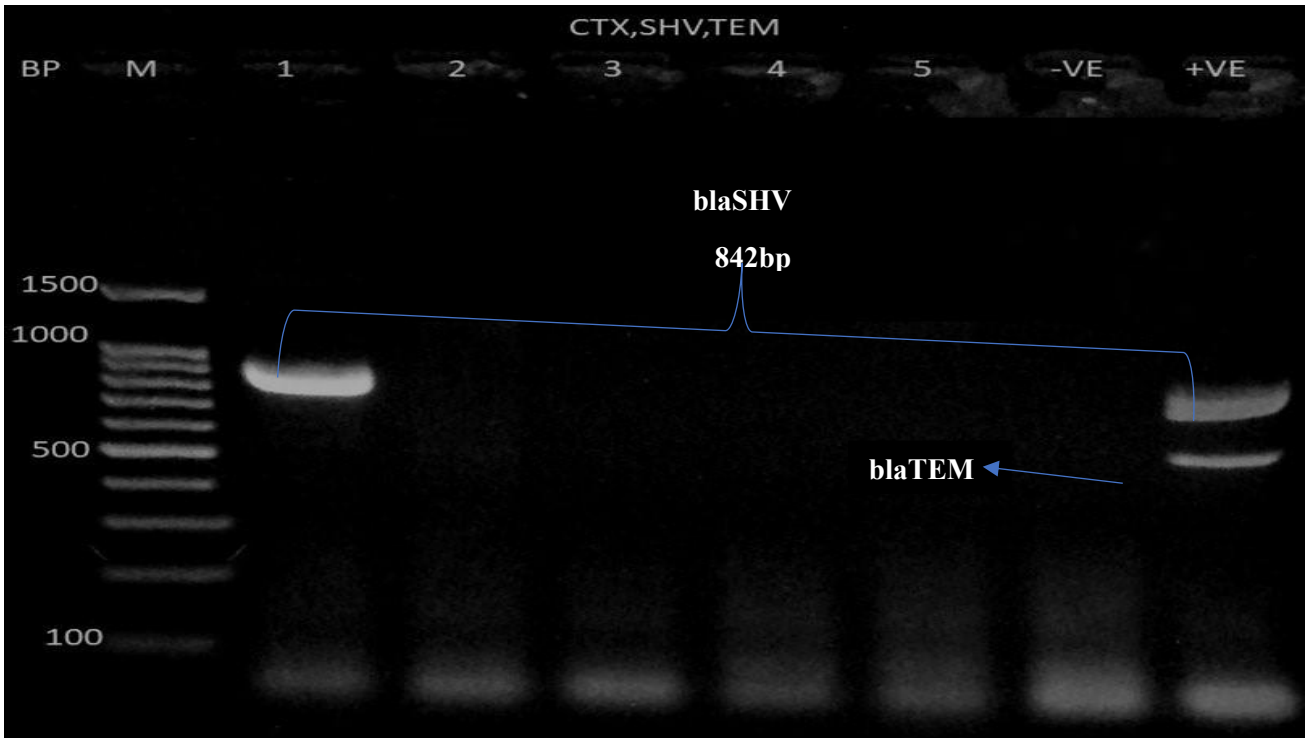

Figure 1: ***Agarose gel image of amplified β-lactamases (BlaSHV) genes in DNA samples from MDR Shigella isolates using multiplex PCR.*** **Lane M is the DNA ladder. Lane 1 shows a BlaSHV band at 800 bp, while lanes 2, 3, 4, and 5 show no detectable bands, indicating negative results for blaTEM, blaSHV, and blaCTX. Lane -ve = negative control & +ve = positive controls (CTX and TEM).**

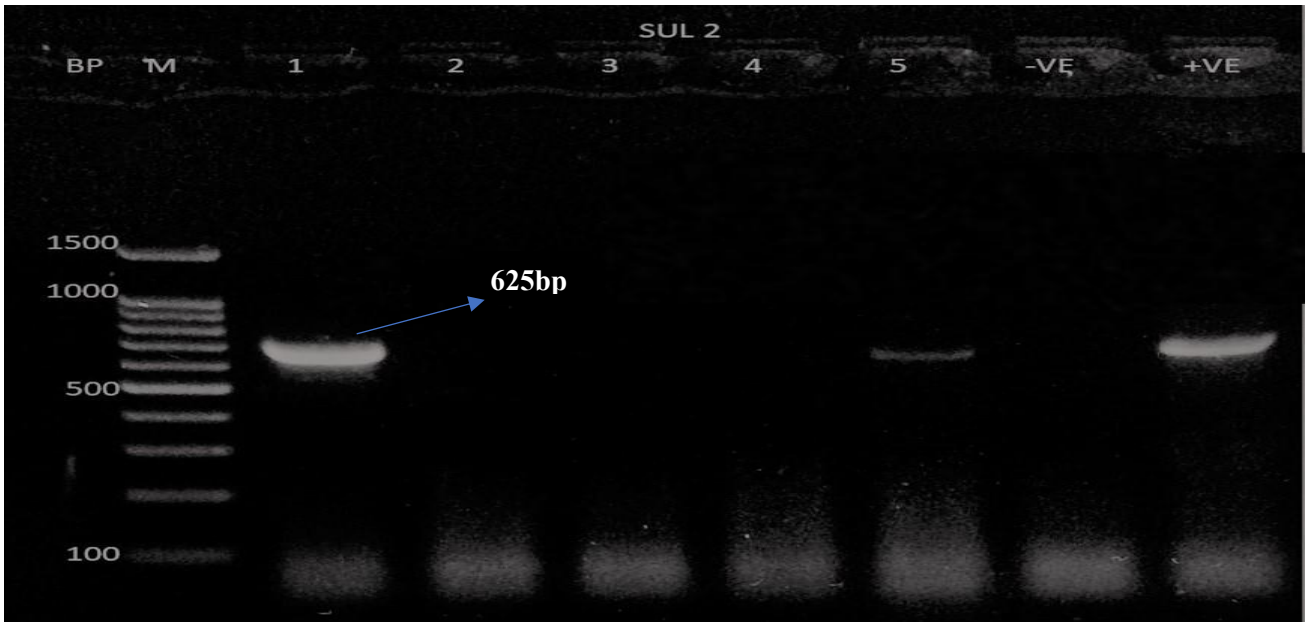

Figure 2**:** ***Agarose gel image of amplified PCR products Sulfonamide (sul2) genes in DNA samples of the MDR Shigella isolates.*** **Lane M: The DNA Ladder, Lanes 1 & 5 show a positive result for the sul2 band at 625 bp. No band is present in Lanes 2, 3, and 4. Lane -ve & +ve are negative & positive controls, respectively.**

The sul1 gene, part of class 1 integrons, was highly frequent, and the sul2 gene is commonly found on both large and small plasmids in clinical *Shigella* isolates. The lack of detection of the genes in other MDR strains could result from various factors, including genetic diversity and variability from mutations, the use of alternative resistance mechanisms such as other Sul genes instead of sul2, and plasmid instability (Deng *et al.,* 2025).

While this Study provides valuable insights on the detection of the resistant genes (Bla_{SHV} and Sul2) in Shigella isolates, the findings should be interpreted carefully due to its limitations, particularly the small sample size and the exclusive reliance on 16S rRNA partial gene sequencing rather than whole genome sequencing for bacterial identification.

CONCLUSION

In conclusion, this Study provides molecular evidence for the occurrence of transferable Bla_{SHV} and Sul2 resistance genes among multidrug-resistant *Shigella flexneri* isolates recovered from Yobe State, Nigeria. Although the analysis was based on a limited number of isolates, the findings suggest the possible circulation of these resistance determinants in the region. Bla_{SHV} and sul2 genes are prevalent and clinically significant resistance markers in MDR *Shigella flexneri*, with PCR serving as a reliable detection method; these underscore the value of broader, systematic molecular surveillance to better characterize their distribution and epidemiological relevance.

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