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Research article

Detection and virulent profiles of multidrug-resistant Shiga toxin-producing *Escherichia coli* (STEC) from horses in Nigeria

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are significant zoonotic pathogens worldwide, causing diseases ranging from diarrhea to hemolytic uremic syndrome (HUS). Ruminants, mainly cattle, are considered the primary reservoirs; however, the role of horses in the epidemiology of STEC remains poorly understood in Nigeria. A total of 231 rectal swabs were collected from horses across selected equine facilities in five Nigerian states. Samples were processed following standard procedures, and isolates were confirmed using the Analytical Profile Index (API 20E) kit. Antibiotic susceptibility testing (AST) was performed using the Kirby-Bauer disk diffusion method with a panel of nine antibiotics, and the isolated *Escherichia* (*E.*) *coli* were classified as sensitive or resistant according to CLSI guidelines. The Multiple Antibiotic Resistance (MAR) index was calculated to assess the risk of contamination. The *E. coli* isolates were screened for selected Shiga toxin genes (*stx1*, *stx2* and *eae*) using polymerase chain reaction (PCR). Fourteen isolates (6%) were identified as Shiga toxin-producing *E. coli*; of these, 78.6% (11/14) expressed at least one virulence gene: 42.9% (6/14) carried *stx2*, 35.7% (5/14) carried *eae*, and no isolate carried *stx1*. All confirmed STEC isolates exhibited multidrug resistance (MDR). The isolates showed complete resistance (100%) to cefuroxime and ciprofloxacin, while all were susceptible to ofloxacin. MAR indices ranged from 0.2 to 0.8, with 77.8% of isolates exhibiting MAR indices ≥0.5. This study reports the detection of MDR STEC in horses in Nigeria, revealing high-level resistance to clinically important antibiotics and significant carriage of virulence genes. The high MAR indices indicate high-risk contamination sources. The detection of MDR STEC in this study underscores a significant health risk to horses, with potential for zoonotic transmission to humans and the environment.

Keywords: Horses, Multidrug resistance, Nigeria, Shiga toxin genes, STEC

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC) is a foodborne pathogen that causes gastrointestinal infections in humans worldwide, leading to kidney failure or even death in severe cases. *Escherichia coli* (*E. coli*) are commensal members of the gastrointestinal tracts of humans and animals such as cattle, bison, and pigs; however, they may acquire Shiga-toxin-encoding phages [1]. STEC are a group of pathogenic bacteria capable of producing potent Shiga toxins (*Stx1* and *Stx2*) that cause diseases in humans and animals, ranging from mild diarrhea to severe hemorrhagic colitis and hemolytic uremic syndrome (HUS) [2, 3]. While ruminants, particularly cattle, are considered the primary reservoirs of STEC [4], recent studies have identified other potential animal hosts [3].

STEC produces Shiga toxins (*Stx*), which are cytotoxic proteins that inhibit protein synthesis in target cells, primarily affecting endothelial cells [5]. The prevalence of STEC in horses appears to be low, with studies in Germany reporting only one positive sample each in horse feces and meat products [6]. Virulence factors, such as Shiga toxins (*Stx1* and *Stx2*), surface adhesins such as intimin (encoded by the *eae* gene), and enterohemolysins (*Ehx* gene), have been detected in various combinations [7, 8, 9]. Characterizing these factors alongside antimicrobial resistance (AMR) patterns revealed that many STEC isolates exhibited multidrug resistance, raising concerns about treatment efficacy [7, 8].

STEC is well-documented in cattle and other livestock; studies specifically focusing on horses remain limited despite the extensive use of horses across sectors such as sports, recreation, agriculture, transportation, and military activities. This gap raises concerns, given the potential for horses to serve as reservoirs for STEC, which can pose risks to both animal and human health [10]. An investigation of *E. coli* strains from donkeys has identified various serotypes, but specific data on STEC in horses is lacking [11]. Other studies have shown that STEC isolates from horses and human cases exhibit resistance to multiple antibiotics, including amoxicillin/clavulanic acid, ceftriaxone, and tetracyclines [12, 13]. A significant proportion of STEC isolates remain susceptible to various antimicrobials [13, 14].

This study aims to effectively detect, isolate, and characterize STEC from horse rectal swabs in Nigeria; to provide valuable insights into AMR patterns of this pathogen in equine populations; to identify selected AMR-

associated genes; and to assess their potential contribution to the overall burden of STEC infections in the local community.

Materials and methods

Ethical approval

The study was conducted in accordance with approved guidelines and standard procedures, and in compliance with the ARRIVE guidelines. It has received ethical approval from the Animal Care and Use Research Committee (ACUREC) of the University of Ibadan (approval number: UI-ACUREC/163-1024/21).

Study area and sample collection

The study was conducted at selected equine farms, stables, and veterinary clinics in 5 localities of Nigeria, i.e., Ogun, Lagos, Abuja, and Kano states, as well as the city of Ibadan. Figure 1 shows the sampling locations. The study employed a cross-sectional design to investigate the prevalence, virulence, and AMR patterns of STEC in rectal samples from horses. Rectal swabs were collected aseptically using sterile swab sticks with gloved hands and placed in bottles containing buffered peptone water. A total of 231 samples were collected (Table 1).

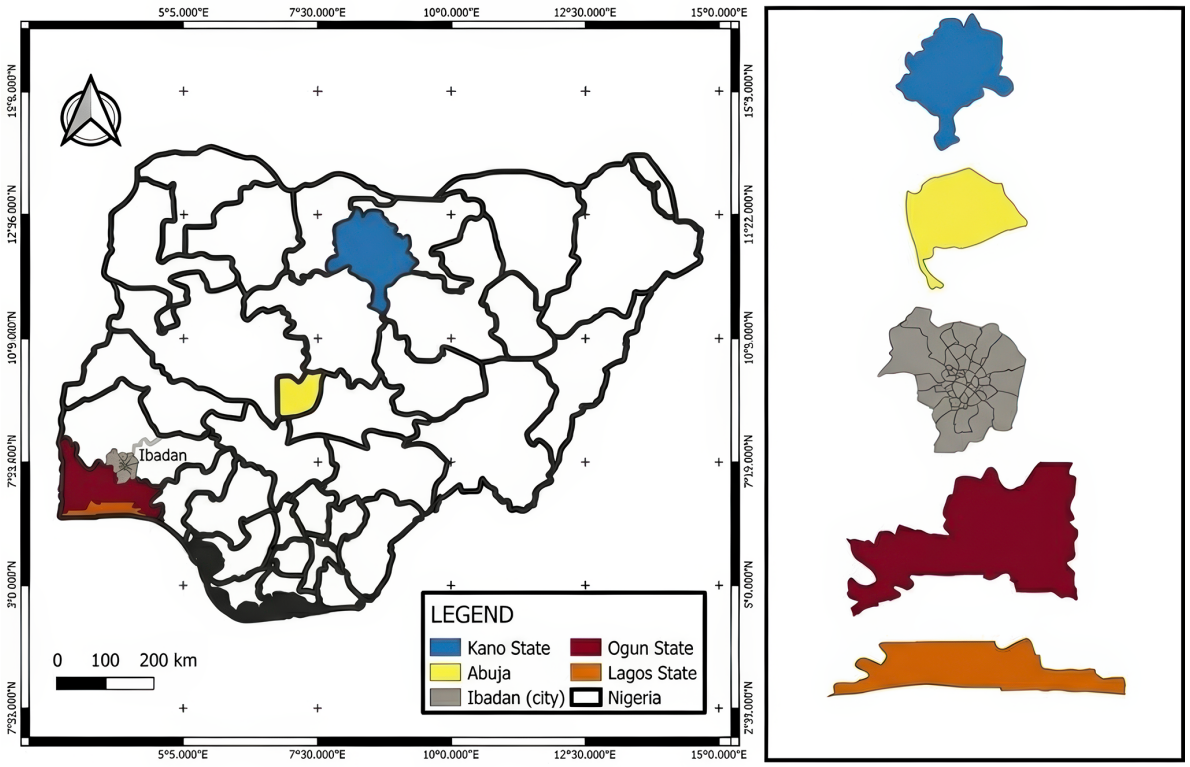

Figure 1: Map of Nigeria showing the geographical location of samples collected from horses and includes 5 states, i.e., Ogun, Lagos, Abuja, and Kano states, as well as the city of Ibadan.

Table 1: Number of samples from various locations in Nigeria used in the current study.

S/N	States (Capital)	Number
1	FCT (Abuja)	32
2	Ogun (Abeokuta)	11
3	Lagos	48
4	Oyo (Ibadan)	90
5	Kano	50
Total		231

Laboratory identification of STEC

The swab sticks were introduced into individual buffered peptone broth (Thermo Scientific, Massachusetts, USA) and incubated for 18 to 24 hours at 37°C. The inoculum was cultured on sorbitol MacConkey (Thermo Scientific, Massachusetts, USA) agar plates. The inoculated plates were incubated at 37°C for 18 to 24 hours. Following incubation, the plates were examined for colonial characteristics. Isolates that produced colorless, transparent colonies (sorbitol MacConkey-negative) were presumptively identified as STEC. These isolates were purified on MacConkey agar (Thermo Scientific, Massachusetts, USA) plates. The inoculated plates were incubated at 37°C for 18 to 24 hours. Following incubation, the plates were examined for colonial characteristics. Isolates that fermented lactose, producing pinkish coloration, were presumptuously identified as *E. coli*. The isolates that were both sorbitol MacConkey negative (colorless, transparent colonies) and lactose fermenters on MacConkey agar (pinkish-colored colonies) were subsequently purified on Eosin Methylene Blue (EMB) Agar (Thermo Scientific, Massachusetts, USA),

picking the pinkish colonies from the MacConkey plates with a sterile wire loop and appropriately inoculating them onto EMB agar plates. The inoculated EMB plates were incubated at 37°C for 18 to 24 hours. Isolates that produced a greenish metallic sheen were identified as suspected *E. coli*, and their identities were further confirmed using the Analytical Profile Index (API 20E) kit [15, 16].

Antibiotic susceptibility test (AST)

The AST was performed using the Kirby-Bauer disk diffusion method, and *E. coli* was classified as sensitive or resistant according to CLSI guidelines [17]. A panel of nine antibiotics was used for the AST (Table 2). Isolates that exhibited resistance to three or more antibiotic classes were classified as multidrug-resistant (MDR) [18]. The multiple antibiotic resistance (MAR) index was determined using the formula: MAR index = a/b, where "a" represents the number of antibiotics to which the isolate showed resistance, and "b" represents the total number of antibiotics tested for susceptibility [19].

Molecular detection of the Shiga toxin genes

DNA was extracted using the standard protocol of Trindade et al. [20]. *E. coli* isolates were screened for selected Shiga toxin genes. PCR was performed using primers for *stx1, stx2,* and *eae,* yielding amplicons of 510 bp, 780 bp, and 570 bp, respectively (Table 3). Amplicons were separated by electrophoresis on a 1.5% agarose gel in Tris-Borate EDTA buffer at 120 volts for 45 minutes. PCR product sizes were estimated by comparing migration distances to bands of the 100 bp molecular weight ladder, visualized on a Trans UV Illuminator after ethidium bromide staining.

Table 2: Antibiotics used for antibiotic susceptibility test (AST) for STEC isolated from horses in Nigeria.

S/N	Code	Name	Concentration	Family
1.	PEN	Penicillin	10µg	Penicillins
2.	APX	Ampicillin	30µg	
3.	CEF	Cefuroxime	30µg	Cephalosporin
4.	TET	Tetracycline	30µg	Tetracyclines
5.	GEN	Gentamicin	10µg	Aminoglycoside
6.	STR	Streptomycin	10µg	
7.	OFL	Ofloxacin	5µg	Fluoroquinolone
8.	LEV	Levofloxacin	5ug	
9.	CPX	Ciprofloxacin	30µg	

Table 3: Primer sequences with the PCR cycling conditions for the detection of *stx1, stx2,* and *eae* genes *in STEC isolates.*

Gene	Primer	Primer sequence 5'-3'	Reference	PCR Amplicon (bp)
Stx1	*Stx1* F	GAGCGAAATAATTTATATGTG	[21]	510
	Stx1 R	TTGATGATGGCAATTCAGTAT		
Stx2	*Stx2* F	CCA TGA CAA CGG ACA GCA GTT		780
	Stx2 R	CCT GTC AAC TGA GCA CTT TGC		
eae	*eae* F	AGGCTTCGTCACAGTTG	[22]	570
	eae R	CCATCGTCACCAGAGGA		

Results

Of the 231 rectal swabs, only 14 isolates (6%) were identified as Shiga toxin producing *E. coli* based on their colony morphology on sorbitol MacConkey agar and biochemical identification using the API 20E. Only 11 of the 14 (78.6%) carried virulence genes; 6 (42.9%) expressed the *stx2* gene; 5 (35.7%) expressed the *eae* gene; and none expressed the *stx1* gene.

Antibiotic susceptibility profile of the *E. coli* isolates

As shown in Table 4, among the 11 isolates with shiga toxin genes, the most resistant antibiotics were cefuroxime and ciprofloxacin, each with 100% resistance. This was followed by 72.73% resistance to penicillin and gentamicin. Tetracycline had a resistance rate of 63.64%, followed by ampicillin at 36.36%. Levofloxacin and streptomycin had resistance rates of 27.27% and 9.09%, respectively. Ofloxacin had the lowest resistance rate, at 0.00%, making it the most effective antibiotic against the *E. coli* isolates. A total of 4 antibiotics (Ampicillin, streptomycin, ofloxacin, and levofloxacin) had resistance below 50%.

MDR pattern and MAR index of the *E. coli* isolates

The MAR indices of the 9 isolates ranged from 0.2 to 0.8, with only two isolates below 0.5. *E. coli* isolates 3, 4, and 5 had the highest MAR index of 0.8, whereas isolates 6 and 9 had the lowest MAR indices of 0.4 and 0.2, respectively. The MAR indices and MDR patterns of the *E. coli* isolates are shown in Table 5.

Table 4: Percentage resistance/sensitivity of the isolates to various antibiotics.

S/N	Antibiotics	% Resistance (%)	% Sensitive (%)
1	Penicillin	72.73 (8/11)	27.27 (3/11)
2	Ampicillin	36.36 (4/11)	63.64 (7/11)
3	Cefuroxime	100.00 (11/11)	0.00 (0/11)
4	Tetracycline	63.64 (7/11)	36.36 (4/11)
5	Gentamicin	72.73 (8/11)	27.27 (3/11)
6	Streptomycin	9.09 (1/11)	90.91 (10/11)
7	Ofloxacin	0.00 (0/11)	100.00 (11/11)
8	Levofloxacin	27.27 (3/11)	72.73 (8/11)
9	Ciprofloxacin	100.00 (11/11)	0.00 (0/11)

Table 5: MDR pattern and MAR indices values of the STEC isolates obtained from horses.

Isolates	Total number of antibiotics tested	Number of antibiotics to which the isolate is resistant	Phenotypic resistance pattern	MAR Index
1	9	5	PEN, CEF, TET, GEN, CPX	0.6
2	9	5	PEN, CEF, TET, GEN, CPX	0.6
3	9	7	PEN, APX, CEF, TET, GEN, LEV, CPX	0.8
4	9	7	PEN, APX, CEF, TET, GEN, LEV, CPX	0.8
5	9	7	PEN, APX, CEF, TET, GEN, STR, CPX	0.8
6	9	2	CEF, CPX	0.2
7	9	6	PEN, CEF, TET, GEN, LEV, CPX	0.7
8	9	6	PEN, APX, CEF, TET, GEN, CPX	0.7
9	9	4	PEN, CEF, GEN, CPX	0.4

Discussion

Horses are generally not considered a major reservoir for STEC. This study newly confirms the presence of MDR STEC infection in horses in Nigeria, with a low detection rate of 5.9%. This study successfully isolated STEC from horses in Nigeria, a public health concern for veterinarians and individuals in close association with horses. Similarly, Pichner *et al.* [6] isolated STEC from horse fecal and meat samples in Germany; STEC was also isolated from horses by Lengacher et al. in Ohio, USA [23]. Thus, a better understanding of Shiga toxin subtypes is needed to properly evaluate the health risks associated with infections [24].

This study had a detection rate of 5.9%; other studies in horses have had lower prevalence, like a 0.4% prevalence found in horses in Ohio, USA, with only a positive STEC isolate in a horse that had had close interaction with at least one ruminant, and was confirmed to have the *stx2* and *eae* genes [23]. In another study in Germany, STEC was isolated from a horse fecal sample and a horse meat sample, both of which carried the *stx2* gene [6].

The higher prevalence of STEC in this study compared with studies in advanced countries may be due to the unhygienic environment in which some horses are raised, the indiscriminate use of antibiotics by farmers, lax biosecurity on farms, a lack of healthy farm practices among some farmers, and limited public health awareness [2]. The environment in which these horses are raised is an important factor. This issue also affects other countries, as Lengacher et al. [23] reported finding only one positive STEC isolate from a horse that had close contact with a ruminant.

A higher prevalence was reported by Reshadi et al. [13] in a study from southeast Iran, who found an STEC prevalence of 29.3% in riding horses. Unlike this report, which had no virulent *stx1* gene profiles, Reshadi et al. [13] reported a higher detection rate of 78.94% for the *stx1* gene.

The *stx*2 and *eae* genes were detected in the isolates in this study. This is of great significance, as the *stx2*-producing strains are associated with severe infections, including hemolytic uremic syndrome (HUS) in humans [25]. In addition, *eae*-positive STEC are often associated with severe intestinal infections [26]. The combination of these genes in the *E. coli* isolates presents a serious virulence profile in horses, which could pose a zoonotic risk to humans.

In this study, the 9 molecularly confirmed STEC isolates were resistant to beta-lactams, tetracyclines, aminoglycosides, and quinolones, meeting the definition of a multidrug-resistant organism [27, 28, 29]. The isolates were generally resistant to beta-lactams, including penicillins and cephalosporins, as well as to tetracyclines. They were also resistant to aminoglycosides and showed 100% resistance to ciprofloxacin and other fluoroquinolones.

This study reports an MAR index of 0.2 to 0.8, with most antibiotics (88.89%) having an MAR index greater than

0.2 and an average MAR index of 0.6, indicating a high risk of contamination due to the indiscriminate use of antimicrobials [30, 31]. Available research shows limited direct analysis of the MAR index in STEC isolates from horses, with many studies offering broader insights into antimicrobial resistance. Reshadi et al. [13] found that 26.15% of *E. coli* isolates from riding horses were multidrug resistant, with the most prevalent resistance against the beta-lactam class (amoxicillin/clavulanic acid (46.2%) and ceftriaxone (38.5%)), as in this study, which showed one of its highest resistances against the beta-lactam class of penicillin and cephalosporin. Maddox et al. [32] reported that 69.5% of horses carried *E. coli* with resistance to at least one antimicrobial. This is a call not only for more research on STEC in horses in Nigeria but also for more research that analyzes the MAR index range for STEC isolates specifically from horses in Nigeria, as MAR indexing is a more easy, rapid, and cost-effective method to analyze bacteria compared to other methods of bacteria tracking [30].

This study highlights the presence of MDR STEC in horses, underscoring the potential risk of human exposure through direct contact. A similar study conducted in the human population showed an antimicrobial resistance pattern similar to that of the penicillin group [32]. In that study, amoxicillin (a penicillin-class antibiotic) showed a high rate of resistance (90.6%). Similarly, Olowe et al. [32] reported low sensitivity to gentamicin. Unlike this study, which had 100% sensitivity to ofloxacin, Olowe et al. [32] reported 20.3% sensitivity to ofloxacin and 75.7% resistance to cefuroxime, compared with the 100% resistance reported in this study.

The advent of antibiotics marked one of the most pivotal breakthroughs in medical science. However, the indiscriminate and unregulated use of antibiotics has resulted in a marked rise in antibiotic resistance and the incidence of resistant infections, thereby severely restricting available therapeutic options [32]. Antibiotic resistance substantially undermines the efficacy of infection treatment, elevating the risk of complications and potentially leading to deadly consequences [32]. The multidrug resistance exhibited by these STEC isolates could present a considerable obstacle to the effective management and control of enteric infections caused by this pathogen. The observed resistance patterns to commonly used antibiotics could be partially attributed to their extensive, prolonged use, unregulated application, and use as prophylactic or metaphylactic agents [32].

Limitations: This study has limitations; we were unable to evenly distribute the sample size across the sampling locations due to limited numbers of horses and limited cooperation from grooms and owners in certain locations. More research on STEC in horses in Nigeria could yield several benefits, particularly for animal health, public health, and food safety.

Conclusion

There had been no prior reports of STEC detection in horses in Nigeria. To the best of our knowledge, this is the first report on the isolation, detection, and MAR index of the virulent profile of STEC in Nigerian horses. The detection of MDR STEC in this study poses a significant health risk to horses and carries the potential for zoonotic transmission to humans. The close interaction between horses and humans, combined with the high prevalence of MDR STEC observed in this study, raises concerns about zoonotic transmission and underscores the need for improved antimicrobial stewardship in equine medicine and enhanced biosecurity in stables and farms where horses are kept. These findings highlight horses as potential reservoirs for MDR STEC, underscoring the need for comprehensive surveillance across multiple species.

Declarations and supplementary information

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Conflict of interest statement: The authors have no conflict of interest.

Ethics statement: The study was conducted in accordance with approved guidelines and methods and was ethically approved by the Animal Care and Use Research Committee (ACUREC) of the University of Ibadan. The assigned ethical approval number is UI-ACUREC/163-1024/21.

Data availability: All data used and analyzed in this study are available upon request from the corresponding author.

Supplementary materials: Not applicable.

Declaration regarding the use of generative AI and AI-assisted technologies in the writing process: Not applicable.

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