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Multiple Antibiotic Resistance Indices of Enteric Bacteria Isolated from *Musca domestica*

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ABSTRACT

The housefly, *Musca domestica*, is a synanthropic pest known to mechanically transmit enteric pathogens by moving between filth and human habitats. In the context of increasing antimicrobial resistance (AMR), flies can act as vectors and environmental reservoirs for multidrug-resistant bacteria, posing a significant public health risk. This study was undertaken to determine the multiple antibiotic resistance indices of enteric bacteria isolated from *Musca domestica*. A total of 100 *M. domestica* specimens were collected from hospital landfill sites, and screened for the presence of enteric bacteria employing standard microbiological technique. *In vitro* technique using disc diffusion method was used for the susceptibility of the isolates to conventional antibiotics. The enteric bacteria isolated from the samples were *Escherichia coli* 0157:H7 strain G5295 (ECOH5), *Escherichia coli* strain 7784 (EC77), *Shigella dysenteriae* strain 1617 (SD16) and *Salmonella enterica* subspecies *enterica* serovar Enteritidis strain EC20121765 (SEE20). The study reveals Musca domestica carries multidrug-resistant enteric bacteria with high Multiple Antibiotic Resistance (MAR) indices, with isolate EC77 showing the highest MAR index, followed by SEE20, SD16, and ECOH5, indicating a significant public health risk ($p < 0.05$). This study has revealed that *Musca domestica* carries multidrug-resistant enteric bacteria (*Escherichia coli*, *Shigella dysenteriae*, and *Salmonella enterica*) with high MAR indices, posing a significant public health risk, emphasizing the need for effective waste management and infection control measures.

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Keywords
Musca domestica, multidrug resistance, MAR index, enteric bacteria.

Introduction

The housefly, *Musca domestica*, is a synanthropic pest that plays a significant role in the transmission of enteric pathogens, including multidrug-resistant bacteria, to humans (Khan *et al*., 2018; Nwakoby *et al*., 2025a; Nwakoby *et al*., 2025b). *M. domestica* is known to mechanically transmit pathogens by moving between filth and human habitats, posing a significant public health risk (Gomes *et al*., 2019; Idigo *et al*., 2025a; Idigo *et al*., 2025b). The increasing prevalence of antimicrobial resistance (AMR) has further exacerbated the problem, with flies acting as vectors and environmental reservoirs for multidrug-resistant bacteria (Adebayo *et al*., 2018; Nwakoby *et al*., 2025c; Nwakoby *et al*., 2025d).

Enteric bacteria, including *Escherichia coli*, *Shigella dysenteriae*, and *Salmonella enterica*, are commonly isolated from *M. domestica* and are known to cause a range of diseases in humans (WHO, 2020; Nwakoby *et al*., 2025e; Nwakoby *et al*., 2025f). The transmission of these pathogens through M. domestica can lead to significant morbidity and mortality, particularly in vulnerable populations such as the elderly, young children, and immunocompromised individuals (Khan *et al*., 2018).

The Multiple Antibiotic Resistance (MAR) index is a useful tool for assessing the antimicrobial resistance patterns of bacteria (Adebayo *et al*., 2018; Idigo *et al*., 2025c; Nwakoby *et al*., 2025g). The MAR index can provide valuable information on the resistance patterns of enteric bacteria isolated, which can inform infection control measures and antibiotic treatment strategies (WHO, 2017; Nwakoby *et al*., 2025h).

This study aimed to determine the multiple antibiotic resistance indices of enteric bacteria isolated from *M. domestica* collected from hospital landfill sites. The findings of this study will contribute to our understanding of the role of *M. domestica* in the transmission of multidrug-resistant enteric bacteria and inform strategies for mitigating this public health risk.

Materials and Methods

Sample Collection, Handling and Transportation

Housefly samples were collected at hospital dumping sites using fly baits (rotten fruits) and perforated plastic containers. The rotten fruits were put into the perforated containers and flies attracted were covered using the cover of the container. The covering of the container and the perforated areas deprived the flies from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection.

Culture and Isolation of Enteric Bacteria

The trapped houseflies were washed using 70% ethanol to remove contaminants. Then, 0.5 g was weighed using an electronic weighing balance (MWP-600) and this was put into a test tube containing 5 ml of normal saline. The flies were smashed using sterile glass rod and a twofold serial dilution was carried out to obtain different concentrations of the samples (2^{-1}, 2^{-2}, and 2^{-3}). One milliliter of the prepared soil samples (2^{-2}) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing MacConkey agar medium (MA/Biotech). All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h as described by Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2018a), Ugwu *et al*. (2025a).

Characterization and Identification of the Isolates
The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010, Iheukwumere *et al*., 2017a; Ugwu *et al*., 2025b), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2018b; Ike *et al*., 2025a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) Dim *et al*. (2025a).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat heat-fixed (Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2025e; Dim *et al*., 2025b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Ike *et al*. (2025b), Iheukwumere *et al*. (2025f).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^{0}C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2018c), Iheukwumere *et al*. (2025g).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Ike *et al*. (2025c) Egbe *et al*. (2025a), Dim *et al*. (2025c).

Indole test: The test was carried out as described by Cheesbrough (2010), Ekechukwu *et al*. (2025a), Egbe *et al*. (2025b), and Obianom *et al*. (2024). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), and Ekechukwu *et al*. (2025b). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025e), Egbe *et al*. (2025c), and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production.

Urease test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), and Ekechukwu *et al*. (2025c). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test.

Methyl red test: The test was carried out as described by Cheesbrough (2010). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2022b), Ekesiobi *et al*. (2025). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at

37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022c), Iheukwumere *et al*. (2024), and Iheukwumere *et al*. (2025k). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022d), Iheukwumere *et al*. (2025l), and Obiefuna *et al*. (2025b). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), Iheukwumere *et al*. (2023a), and Iheukwumere *et al*. (2023b). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025m; Iheukwumere *et al*., 2022e; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94℃ for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72℃ for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2022f; Iheukwumere *et al*., 2025n; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2022g; Nwike *et al*., 2017).

Determination of Prevalence of the Isolates in the Studied Samples

The occurrences of different strains of the bacterial isolates associated with the s samples were counted and recorded according to the method described in the study published by Iheukwumere *et al*. (2018). The number of the occurrences of the predominant bacteria were counted, and their percentages of occurrences were appropriately calculated and recorded.

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* activity of conventional antibiotics against the isolates using disc diffusion method**

The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37˚C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Iheukwumere *et al*. (2018).

Statistical Analysis

The data generated were expressed in percentages and Tables. The significance of the study was determined using Analysis of Variance (ANOVA) at a 95% confidence level. Post-hoc analysis was carried out using Tukey's HSD (Honestly Significant Difference) test from IBM SSPS version 30 (Amadi *et al*., 2017; Iheukwumere *et al*., 2017e; Manasseh *et al*., 2025a; Manasseh *et al*., 2025b; Iheukwumere *et al*., 2022a and Iheukwumere *et al*., 2025o).

Results

The isolates (B1, B2, C1, C2) exhibited distinct cultural and morphological characteristics. B1 and B2 appeared red and pink on MacConkey agar, respectively, indicating lactose fermentation, while C1 and C2 appeared colorless, indicating non-lactose fermentation. All isolates were Gram-negative rods, motile (except C1), and positive for catalase and methyl red (MR) tests, but negative for oxidase, urease, and Voges-Proskauer (VP) tests. Biochemical tests identified the isolates as E. coli (B1, B2), Shigella (C1), and Salmonella (C2).

The DNA concentrations of the isolates ranged from 101.30 ng/µL (C1) to 119.10 ng/µL (C2). The 260/280 ratios

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indicated pure DNA (1.81-1.84). Molecular analysis confirmed the isolates as: B1: Escherichia coli O157:H7 strain G5295 (ECOH5), B2: Escherichia coli strain 7784 (EC77), C1: Shigella dysenteriae strain 1617 (SD16), C2: Salmonella enterica subspecies enterica serovar Enteritidis strain EC20121765 (SEE20). The isolates were detected in Musca domestica specimens: EC77: 41.50% (most prevalent), SEE20: 22.00%, SD16: 18.50%, ECOH5: 11.00% The isolates showed varying susceptibility patterns: EC77: 73.49% susceptible, SD16: 56.76% susceptible, SEE20: 38.64% susceptible, ECOH5: 36.36% susceptible. The MAR indices indicated high multidrug resistance: SEE20: highest MAR index (0.90-1.00), EC77: moderate MAR index (0.20-0.80), SD16 and ECOH5: lower MAR indices. The study found significant differences ($p < 0.05$) in the MAR indices and antibiotic resistance patterns among the isolates. The study identified multidrug-resistant enteric bacteria (*E. coli*, *Shigella dysenteriae*, and *Salmonella enterica*) in *Musca domestica*, with high MAR indices, posing a significant public health risk.

Table 1: Cultural, morphological characteristics and biochemical characteristics of the isolates

Characteristics	**B1**	**B2**	**C1**	**C2**
Appearance	Red on MA	Pink on MA	Colourless on MA	Colourless on MA
Margin	Smooth	Smooth	Smooth	Smooth
Edge	Smooth	Smooth	Smooth	Smooth
Gram Reaction	-	-	-	-
Shape	Rods	Rods	Rods	Rods
Motility	+	+	-	+
Catalase	+	+	+	+
Oxidase	-	-	-	-
Indole	+	+	-	-
MR	+	+	+	+
VP	-	-	-	-
Citrate	-	-	-	+
Urease	-	-	-	-
H2S	-	-	-	+
Casein	-	-	-	-
Gelatin	-	-	-	-
Glucose	+	+	+	+
Maltose	+	+	-	+
Sucrose	+	+	-	+/-
Lactose	+	+	-	-
Xylose	+	+	-	+
Mannitol	+	+	-	+
Arabinose	+	+	-	+/-
Sorbitol	-	+	-	+/-
Inositol	+/-	+/-	-	-
Dulcitol	+/-	+/-	-	+
Bacterium	*E. coli*	*E. coli*	*Shigella*	*Salmonella*

Table 2: Nucleic acids extracted from the isolates

Isolate	Conc µg/nL	280nm	260nm	260/280
B1	102.60	1.7210	3.1320	1.82
B2	112.40	1.7430	3.1900	1.83
C1	101.30	1.7180	3.1100	1.81
C2	119.10	1.7630	3.2440	1.84

Table 3: Molecular identities of the isolates

Parameter	B1	B2	C1	C2
Max score	38284	38278	2069	26613
Total score	38284	38278	2069	26613
Query cover (%)	100	100	100	100
E-value	0.0	0.0	0.0	0.0
Identity	100	100	100	100
Accession number	CP038346	CP018247	CP006736	CP007429
Description	*Escherichia coli* 0157:H7 strain G5295 (ECOH5) chromosome, complete genome	*Escherichia coli* strain 7784 (EC77) complete genome	*Shigella dysenteriae* Strain 1617 (SP16) complete genome	*Salmonella enterica* subspecies *enterica* serovar Enteritidis strain EC20121765 (SEEC20) chromosome

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Table 4: Occurrences of the isolates

Isolate	Number of *Musca domestica*	Positive (%)	Negative (%)
ECOH5	200	22 (11.00)	178 (89.00)
EC77	200	83 (41.50)	117 (58.50)
SD16	200	37 (18.50)	163 (81.50)
SEE20	200	44 (22.00)	156 (78.00)
Total	800	186 (23.25)	614 (76.75)

Table 5: Susceptibility of the isolates to conventional antibiotics

Isolate	Number	Susceptible strain (%)	Resistant strain	Implicated Antibiotics
ECOH5	22	8(36.36)	14 (63.64)	S, PN, SXT, AU, CN, CEP, AMX
EC77	83	61 (73.49)	22 (26.51)	S, PN, SXT, AU, CN, CEP, AMX
SD16	37	21 (56.76)	16 (43.24)	S, PN, SXT, AU, CN, CEP, AMX
SEE20	44	17 (38.64)	27 (61.36)	S, PN, SXT, AU, CN, CEP, AMX
Total	186	107 (57.53)	79 (42.47)	S, PN, SXT, AU, CN, CEP, AMX

Table 6: Multiple antibiotic resistance (MAR) indices of the isolates

MAR Index	ECOH5 (14)	EC77 (22)	SD16 (16)	SEE20 (27)
0.10	0 (0.0)	8 (36.36)	4 (25.00)	9 (33.33)
0.20	2 (14.29)	1 (4.55)	0 (0.00)	0 (0.00)
0.30	0 (0.00)	4 (18.18)	2 (12.50)	6 (22.22)
0.40	3 (21.43)	0 (0.00)	1 (6.25)	3 (11.11)
0.50	2 (14.29)	5 (22.73)	1 (6.25)	4 (14.81)
0.60	0 (0.00)	1 (4.55)	3 (18.75)	1 (3.70)
0.70	3 (21.43)	2 (9.09)	5 (31.25)	0 (0.00)
0.80	1 (7.14)	1 (4.55)	0 (0.00)	0 (0.00)
0.90	3 (21.43)	0 (0.00)	0 (0.00)	3 (11.11)
1.00	0 (0.00)	0 (0.00)	0 (0.00)	1 (3.70)

Discussion

The cultural, morphological, and biochemical characteristics of the isolates in this study are consistent with previous reports, which describe *E. coli*, *Shigella dysenteria*e, and *Salmonella enterica* as Gram-negative rods that are positive for catalase and methyl red tests (Adebayo *et al.*, 2018; Khan *et al*., 2018; Idigo *et al*., 2025d; Nwakoby *et al*., 2025i). The molecular identities of the isolates, confirmed by 16S rRNA gene sequencing, are also consistent with previous studies that have reported the presence of these pathogens in *Musca domestica* (Gomes *et al*., 2019; Nwakoby *et al*., 2025j; Nwakoby *et al*., 2025k; Idigo *et al*., 2025e).

The prevalence of the isolates in *Musca domestica* specimens in this study is similar to previous reports, which have shown that *E. coli* is the most prevalent enteric pathogen in houseflies, followed by *Salmonella* and *Shigella* species (Khan *et al*., 2018; Adebayo *et al.*, 2018; Nwakoby *et al*., 2025l; Nwakoby *et al*., 2025m; Nwakoby *et al*., 2025n). The high prevalence of EC77 (41.50%) in this study is concerning, as *E. coli* O157:H7 is a major cause of foodborne illness worldwide (WHO, 2020).

The antibiotic susceptibility patterns of the isolates in this study are also consistent with previous reports, which have shown that enteric pathogens isolated from *Musca domestica* are often resistant to multiple antibiotics (Gomes *et al*., 2019; Adebayo *et al.*, 2018; Nwakoby *et al*., 2025o; Nwakoby *et al*., 2025p). The high MAR indices observed in this study, particularly for SEE20 (0.90-1.00), indicate a significant public health risk, as these pathogens can spread antibiotic resistance genes to other bacteria (Khan *et al*., 2018). The findings of this study highlight the importance of *Musca domestica* as a vector of multidrug-resistant enteric pathogens and emphasize the need for effective waste management and infection control measures to prevent the spread of these pathogens (WHO, 2017).

Conclusion

This study has shown that *Musca domestica* carries multidrug-resistant enteric bacteria, including *E. coli*, *Shigella dysenteriae*, and *Salmonella enterica*, with high MAR indices, posing a significant public health risk.

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Conflict of interests

The authors declare that they have no conflict of interests.

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