Characterizing Shigella Drug Resistance in Animal-Based Foods: Implications for Public Health

ABSTRACT

The prevalence of foodborne pathogens has increased in recent years, posing a significant threat to both food safety and human health. In developing countries, the practice of sanitation and hygiene is very poor, which is a major concern. In connection with this issue, a study has been conducted to assess the presence of *Shigella* in meat, chicken, fish, prawn, and milk samples collected from local vendors across the city of Mumbai. *Shigella* was isolated using streaking and spreading methods, and several biochemical tests were performed to confirm its presence. Additionally, these samples were tested for drug resistance against various antibiotics, including Ampicillin, Ciprofloxacin, Cefoperazone, Chloramphenicol, Erythromycin, Gentamycin, Nalidixic acid, Tetracycline, Trimethoprim, and Ofloxacin. The study helped us uncover the contamination rates present in the different samples, which could lead to severe health threats. Proper sanitary measures are urgently needed to address this issue."

INTRODUCTION

Shigella, a Gram-negative bacterium, is the culprit behind severe intestinal infections known as bacillary dysentery or shigellosis. Shigellosis, a highly problematic and contagious infectious disease, is caused by various species of *Shigella* and is primarily transmitted through contaminated food and water [1].The physical characteristics of *Shigella* include straight rods measuring 1–3 × 0.7–1.0 µm. These rods conform to the expansive definition of the *Enterobacteriaceae* family and are distinguished by the presence of the enterobacterial common antigen. Gram-negative, nonmotile, and lacking pigmentation, *Shigella* exhibits facultative anaerobic behaviour, engaging both respiratory and fermentative metabolic pathways[3].

While positive catalase activity is a common trait, exceptions exist, such as *Shigelladysenteriae*. Oxidase negativity defines these chemoorganotrophic organisms, which ferment sugars without gas production, with a few exceptions that generate gas. Certain substances like salicin, adonitol, and myo-inositol remain unfermented [9].*Shigella sonnei* strains exhibit lactose and sucrose fermentation after extended incubation, while other species do not metabolize these substances conventionally. Citrate, malonate, or sodium acetate (exceptions in *ShigellaS.flexneri* concerning sodium acetate) are not utilized as sole carbon sources. Growth is not sustained in KCN, and hydrogen sulphide (H2S) production is absent. *Shigella*, categorized within the Gammaproteobacteria taxonomic group by 16S rDNA sequencing, serve as intestinal pathogens causing bacillary dysentery [16].

Shigellosis infection involves the invasion of epithelial cells in the colon, ileum, and rectum. *Shigella* species cause approximately 125 million diarrheal episodes annually, affecting individuals of all age groups but being more commonly reported in vulnerable populations. Current methods for identifying *Shigella* are labour-intensive, time-consuming, and error-prone, partly because of its close resemblance to Entero-Invasive *Escherichia coli* (EIEC), with challenging serotype interpretation for inexperienced users. *Shigella* comprises four species: *S.flexneri*, *S. sonnei*, S. *dysenteriae*, and *S. boydii*, with *S. boydii* having the highest number of serotypes[18]. Some strains, particularly *S. boydii*, exhibit resistance to antibiotics like azithromycin and ciprofloxacin. The pathogenic process of *S.flexneri* involves bile and glucose as signalling molecules for biofilm formation and increased virulence. Upon reaching the colon's epithelial lining, *S.flexneri* interacts with specialized microfold cells (M-cells), facilitating invasion of colonic epithelial cells[5].

In contrast, *Shigelladysenteriae* utilizes an alternative invasion mechanism by releasing AB5 toxins. The Shiga toxin disrupts protein synthesis in host cells by permanently deactivating ribosomes. The rise of antibiotic-resistant strains of *S. dysenteriae* globally poses significant concerns in treating shigellosis. Understanding *Shigella* pathogenicity mechanisms is crucial for developing prevention strategies against shigellosis, especially with the emergence of bacterial pathogens resistant to multiple drugs. The presence of R factors (plasmids carrying resistant genes) plays a crucial role in the development of new serotypes promoting antibacterial resistance[10].*Shigella*, as an enteropathogenic with high virulence, adeptly operates in the human intestine. The type three secretion system, initiating diseases, encompasses a diversity of T3ss effectors targeting cellular functions. Although antibiotics like Tetracycline, ampicillin, chloramphenicol, nalidixic acid, and trimethoprim-sulfamethoxazole were once used to treat shigellosis, species of *Shigella* have gradually developed resistance to these drugs[17].

The recent advancement of next-generation sequencing has significantly streamlined 16S rRNA gene sequencing. Benchtop sequencers now empower smaller laboratories to execute 16S rRNA sequencing on-site efficiently. Environmental DNA undergoes emulsion PCR, and resulting sequences are loaded onto a disposable chip for sequencing. The obtained sequences

are filtered, and barcodes discern sample origins. Further analysis using publicly accessible tools, including a taxonomy-finding algorithm, demonstrates the simplicity, cost-effectiveness, and accessibility of this approach for smaller laboratories during the ongoing genomic revolution[26].

This study aims to assess the prevalence and resistance profiles of multi-drug resistant *Shigella* strains from various sources such as meat, fish, and eggs. Study seeks to determine drug resistance patterns and the extent of spread of multi-drug resistant *Shigella* strains across diverse sources, contributing valuable insights into combating this global health concern [3,11]. In developing nations, *Shigella* infections significantly contribute to morbidity and mortality, especially in children with diarrhoea. To address the imperative need for comprehensive epidemiological data at the country level, molecular characterization of *Shigella* species genetic determinants is essential. Recent advancements in *Shigella* research have deepened our understanding of the molecular mechanisms, predominantly using *S.flexneri* as a model[24]. *Shigella*'s virulence strategy goes beyond host-cell invasion, involving survival in harsh gastrointestinal conditions, competition with the host microbiota, and traversing the intestinal mucus layer. Despite these crucial aspects, the diversity of virulence strategies among *Shigella* species remains underexplored, posing a gap in understanding global epidemiology and validating vaccine candidates. High-throughput screening and sequencing methods facilitate complex comparative studies. While *Shigella* research has focused on direct interactions with host cells, molecular characterization has centredon *S.flexneri*. Virulence mechanism\s have been predominantly elucidated using *S.flexneri*, despite other groups exhibiting high prevalence worldwide[8].

Shigella remains a significant threat to childhood health, evidenced by the Global Enteric Multicentre Study (GEMS) identifying it among top pathogens causing severe diarrhoea in Sub-Saharan Africa and Asia. Geographical stratification based on economic development highlights*S.flexneri* prevalence in the developing world and increasing *S. sonnei* rates with economic development. Rising *S. sonnei* cases challenge expectations, with potential explanations including cross-protective immunity, increased survival in Acanthamoeba as a reservoir, and enhanced antibiotic resistance[3]. The counterintuitive rise of *S. sonnei* amid improved sanitation raises questions about global public health concerns and vaccine development impacts. Rapid antibiotic resistance acquisition, especially in *S. sonnei,* poses a global health challenge. *S. sonnei* ability to share resistance plasmids emphasizes the urgent need for measures to prevent global spread, while antibiotic resistance in *S.flexneri* underscores the need for targeted surveillance and localized efforts. Changing dynamics, such as the disappearance of Sd1 and the shift from S.*flexneri* to *S. sonnei*, pose unanswered questions. Research focusing on early establishment and colonization steps may provide insights into epidemiological observations [17, 22].The molecular basis of *Shigella*'s colon-specific invasion remains unknown, necessitating further investigation into perturbations induced by *Shigella* invasion and their impact on protective barriers and the inflammatory response.

MATERIALS AND METHODS

Methods of isolation and identifications

The methods employed for isolation and identification were meticulous and rigorous. A total of 112 meat samples, comprising 39 from meat, 34 from fish, 17 from chicken, 12 from prawns, and 10 from milk, were meticulously collected in UV sterilized zipped plastic bags and promptly transported to the laboratory within iceboxes [7]. Stringent measures were undertaken to prevent any possibility of cross-contamination during sampling, including the utilization of sterile gloves and forceps.Sampling was carried out from randomly scattered meat shops and dairies across various locations in Mumbai, with only one type of meat collected per day to ensure sample purity. For the isolation from meat samples, each 25-gram sample of chicken and meat was homogenized in 225 ml of Peptone Water (PW) and incubated overnight at 37°C. The pre-enriched culture was then inoculated into Selenite F Broth (SB) and Rappaport Vassiliadis Soya (RVS) Broth, followed by a 24-hour incubation period at 37°C. For prawn and fish samples, 250 gm of the sample was aseptically blended. From this blend, 10 gm samples were taken and mixed with 90 ml of sterile normal saline (0.9% w/v NaCl) solution in a conical flask to create a homogeneous suspension. The samples were serially diluted, thoroughly mixed using a Vortex-mixture, and analysed for microbial contamination. For milk samples, 25 millilitres of raw cow milk were mixed with Shigella Broth (Merck, Germany) fortified with 0.5 micrograms per millilitre of novobiocin. The mixture was homogenized for 1 minute at a speed of 320 revolutions per minute within a Stomacher Bag Mixer and then incubated anaerobically overnight at 42 degrees Celsius. Subsequently, 0.1 millilitre aliquots of the enriched samples were inoculated onto MacConkey agar plates (HI Media) and subjected to anaerobic incubation at 42 degrees Celsius for 48 hours [10, 18].

Characterization and Biochemical Tests

Subsequent steps involved the direct inoculation of a loop from the enriched cultures onto various agar mediums, including Shigella Selection (S-S) agar, MacConkey agar, and xylose-lysine-deoxycholate (XLD) agar, followed by incubation at 37°C for 24 hours. Bacteria cultured in Selenite F Broth (SB) and Rappaport Vassiliadis Soya (RVS) Broth were streaked onto MacConkey agar to differentiate between lactose and non-lactose fermenters. Non-lactose fermenting bacteria were further streaked onto XLD and S-S agar. Colonies exhibiting specific characteristics—such as a convex colourless appearance on

MacConkey agar and a colourless appearance on S-S and XLD agar—were considered presumptive Shigella colonies and subjected to further biochemical confirmation.

The biochemical assessment of all Salmonella isolates involved a series of tests, including indole (I), methyl red (M), Voges-Proskauer (Vi), citrate (C), triple sugar iron (TSI), and urease tests, following the methodology outlined by Ewing. Specifically, colonies displaying the characteristic Salmonella-specific IMViC pattern (-+-+) underwent further inoculation on TSI slants. Here, colonies showing an alkaline slant (pink) and acidic butt (yellow) on TSI, with or without hydrogen sulphide (H2S) production (indicated by blackening), were subsequently evaluated for urease production on urea agar slants. Those isolates lacking urease activity were classified as biochemically confirmed Salmonella isolates. The process of biochemical confirmation entailed a battery of tests, including TSI agar, lysine iron agar (LIA), methyl red, Voges-Proskauer (VP) broth, indole test, urea agar (UA), Simmon's citrate agar (SCA), and a motility test. Following confirmation, serotyping of the isolates was conducted through slide agglutination assays utilizing commercially available kits. For long-term preservation, all bacterial isolates were stored at -80°C in tryptic soy broth (TSB) supplemented with 25% glycerol, ensuring viability for subsequent analyses.

16S rRNA and Molecular Techniques

The molecular identification of the isolates was carried out by amplifying and sequencing the 16S ribosomal RNA gene using universal primers 8F and 907R. The genomic DNA of the isolates was extracted using Cetyl trimethyl ammonium bromide (CTAB) buffer with slight modifications to the method by Chapela et al. The PCR reaction mixture consisted of 2.5 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM primers, 2.5 units of Taq DNA polymerase, 50 ng of template DNA, and 1X buffer. The PCR conditions included an initial denaturation at 96°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension step at 72°C for 5 minutes. The resulting amplicons were analyzed on agarose gel to assess amplification and purity, then purified using a PCR purification kit and subjected to Sanger sequencing. The obtained FASTA file from sequencing was aligned with a nucleotide database using the BLAST Tool, and the gene sequences were submitted to GenBank for further analysis and documentation. [26].

Antimicrobial resistance profiling of the isolates

The susceptibility of bacterial isolates to drugs was assessed using a Kirby–Bauer disk diffusion assay, in accordance with the established standards and interpretive criteria outlined by the Clinical and Laboratory Standards Institute (CLSI). All *Shigella* isolates underwent examination to determine their susceptibility or resistance to antimicrobial drugs via disc diffusion technique. Antibiotic discs containing ofloxacin (OF, 5 µg), ciprofloxacin (CIP, 5 µg), Gentamycin (GEN, 30 µg), Erythromycin (E, 15 µg), Nalidixic acid (NA, 30 µg), Ampicillin (AMP, 10 µg), Chloramphenicol (C, 30 µg),Cefoperazone (CPZ, 75 µg),Tetracycline (TE, 30 µg), and Trimethoprim (TR, 5 µg) (procured from HI Media, India) were utilized(4). The diameters of zones exhibiting complete inhibition were measured and compared with the zone size interpretation chart provided by the supplier, and subsequently categorized as sensitive, intermediate, or resistant. The zone of acceptance of each antibiotic is mentioned. ofloxacin (OF, S-16mm or more, I-13mm-15mm, R-12mm or less), ciprofloxacin (CIP, S-26mm or more, I-22mm-25mm, R-21mm or less), Gentamycin (GEN, S-15mm or more, I-13mm-14mm, R-12mm or less), Erythromycin (E, S-23mm or more, I-14mm-22mm, R-13mm or less), Nalidixic acid (NA, S-19mm or more, I-14mm-18mm, R-13mm or less), Ampicillin (AMP, S-17mm or more, I-14mm-16mm, R-13mm or less), Chloramphenicol (C, S-18mm or more, I-13mm-17mm, R-12mm or less),Cefoperazone (CPZ, S-21mm or more, I-16mm-20mm, R-15mm or less), Tetracycline (TE, S-15mm or more, I-12mm-14mm, R-11mm or less), and Trimethoprim (TR, S-16mm or more, I-11mm-15mm, R-10mm or less). Additionally, the multiple antibiotic resistance (MAR) index was calculated for all *Shigella* isolates following the methodology prescribed by the Clinical and Laboratory Standards Institute (CLSI), employing the formula a/b, where "a" represents the number of antibiotics to which an isolate demonstrated resistance, and "b" signifies the number of antibiotics to which the isolates were exposed. The antibiotic susceptibility test (AST) for all identified isolates was conducted using ten antibiotics via the Kirby Bauer disc diffusion method. Interpretation of sensitivity (S), intermediate sensitivity (I), and resistance (R) was performed in accordance with CLSI guidelines. The selection of antibiotics was based on those commonly used in India and those recommended by the World Health Organization (WHO) for routine integrated antimicrobial resistance monitoring. Quality control was ensured by employing *Shigella* 23354 culture.

RESULTS AND DISCUSSION

The primary source of dietary protein encompasses meat, chicken, fish, prawn, and milk. Mumbai boasts a considerable consumer base for dairy, poultry, and meat products. The study investigates the safety of meat, poultry, and dairy products in Mumbai amidst the rise of multi-drug resistant organisms[2]. In densely populated areas with a significant consumer base for these products, people often buy fresh items from local vendors due to convenience and cost. However, concerns arise about the hygiene standards in these establishments. Poor personal hygiene, unclean environments, and potential faecal contamination in shops can lead to the spread of pathogens and diseases. Antibiotic overuse in animal feed worsens

antibiotic resistance issues[19]. Therefore, the study aims to assess the prevalence of foodborne pathogens, particularly *Shigella*, and their antibiotic resistance levels in various locations across Mumbai.

Shigella isolation was achieved using the spread and streaking method on XLD agar media. The isolated bacteria displayed a rod-like morphology and were Gram-negative. Biochemical tests were conducted on isolates obtained from various samples To confirm the identification of the isolates as *Shigella*, standard reference results were followed. The catalase test produced a positive result, the gelatin test yielded a negative result, and the TSI slant test indicated a yellow coloration with no hydrogen sulphide (H2S) or gas production as shown in Table 1.

In our study, the prevalence rate of *Shigella spp*. was higher in meat (36.67%) than in fish (16.67%), prawn (16.67%), milk (20%)and chicken (10%) as shown in Figure 1. In a study conducted in Egypt, Ahmed and Shimamoto (2014) found a higher occurrence of *Shigella spp.* in meat samples (2.0%) compared to dairy products (1.4%). Conversely, a recent study in Iran by Shahin et al. (2019) revealed a higher prevalence of Shigella spp. in vegetables (2.2%) than in ready-to-eat (2.0%) and meat samples (0.8%). Pakbin et al (2021) indicated higher prevalence of *Shigella spp.* in vegetable salads(3.20%) than in raw cow's milk (0%) and meat samples. Due to their close genetic proximity, humans and apes are recognized as the main reservoirs of *Shigella spp.*[18]. Consequently, there is a risk of *Shigella* contamination in various food items such as raw vegetables, vegetable salads, meat, and dairy products, particularly when handled by food preparers or processed using equipment tainted with the bacteria [1].

Table 1 Biochemical Characterization of the Shigella isolates from different animal-based food samples.

Sr no.	Isolate	Morphology	Catalase	Citrate	Lysine	Ornithine	Motility	ONPG	Mannitol	Gelatine	H2S production	Gas production
1	P4	Rod	+	-	-	+	-	-	+	-	-	-
2	P8	Rod	+	-	-	+	-	-	+	-	-	-
3	P10	Rod	+	-	-	-	-	-	+	-	-	-
4	P11	Rod	+	-	-	-	-	-	+	-	-	-
5	P12	Rod	+	-	-	-	-	-	+	-	-	-
6	M18	Rod	+	-	-	-	-	-	+	-	-	-
7	M19	Rod	+	-	-	-	-	-	+	-	-	-
8	M20	Rod	+	-	-	-	-	-	+	-	-	-
9	M21	Rod	+	-	-	-	-	-	+	-	-	-
10	M22	Rod	+	-	-	-	-	-	+	-	-	-
11	M23	Rod	+	-	-	-	-	-	+	-	-	-
12	MT2	Rod	+	-	-	-	-	-	+	-	-	-
13	MT3	Rod	+	-	-	-	-	-	+	-	-	-
14	MT4	Rod	+	-	-	-	-	-	+	-	-	-
15	MT5	Rod	+	-	-	-	-	-	+	-	-	-
16	MT6	Rod	+	-	-	-	-	-	+	-	-	-
17	MT7	Rod	+	-	-	-	-	-	+	-	-	-
18	MT17	Rod	+	-	-	-	-	-	+	-	-	-
19	MT19	Rod	+	-	-	-	-	-	+	-	-	-
20	MT20	Rod	+	-	-	-	-	-	+	-	-	-
21	MT21	Rod	+	-	-	-	-	-	+	-	-	-
22	MT32	Rod	+	-	-	-	-	-	+	-	-	-
23	C22	Rod	+	-	-	-	-	-	+	-	-	-
24	C23	Rod	+	-	-	-	-	-	+	-	-	-
25	C17	Rod	+	-	-	-	-	-	+	-	-	-
26	F22	Rod	+	-	-	-	-	-	+	-	-	-
27	F27	Rod	+	+	-	-	-	-	+	-	-	-
28	F34	Rod	+	-	-	-	-	-	+	-	-	-

29	F41	Rod	+	+	-	-	-	+	+	-	-	-
30	F50	Rod	+	+	-	-	-	-	+	-	-	-

Table 2 The molecular characterization of the isolates using 16S rRNA Technique.

SR NO.	SAMPLE	GENBANK ID	BACTERIA NAME
1	P4	PP647807	*Shigella flexneri*
2	P8	PP647808	*Shigella flexneri*
3	P10	PP647809	*Shigella dysenteriae*
4	P11	PP647810	*Shigella flexneri*
5	P12	PP647811	*Shigella dysenteriae*
6	M18	PP647812	*Shigella dysenteriae*
7	M19	PP647813	*Shigella flexneri*
8	M20	PP647814	*Shigella flexneri*
9	M21	PP647815	*Shigella dysenteriae*
10	M22	PP647816	*Shigella flexneri*
11	M23	PP647817	*Shigella dysenteriae*
12	MT2	PP647818	*Shigella dysenteriae*
13	MT3	PP647819	*Shigella flexneri*
14	MT4	PP647820	*Shigella dysenteriae*
15	MT5	PP647821	*Shigella flexneri*
16	MT6	PP647822	*Shigella flexneri*
17	MT7	PP647823	*Shigella dysenteriae*
18	MT17	PP647824	*Shigella dysenteriae*
19	MT19	PP647825	*Shigella flexneri*
20	MT20	PP647826	*Shigella dysenteriae*
21	MT21	PP647827	*Shigella flexneri*
22	MT32	PP647828	*Shigella dysenteriae*
23	C22	PP647829	*Shigella flexneri*
24	C23	PP647830	*Shigella dysenteriae*
25	C17	PP647831	*Shigella dysenteriae*
26	F22	PP647832	*Shigella dysenteriae*
27	F27	PP647833	*Shigella flexneri*
28	F34	PP647834	*Shigella dysenteriae*
29	F41	PP647835	*Shigella flexneri*
30	F50	PP647836	*Shigella dysenteriae*

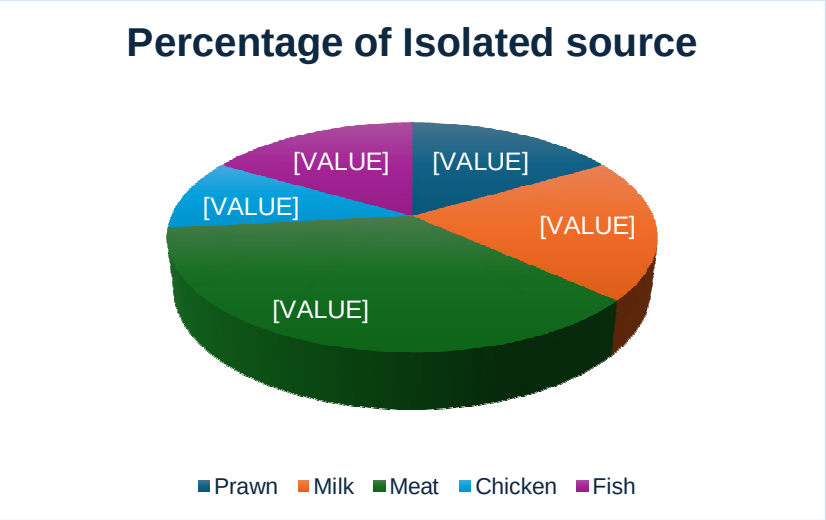

Figure 1 Distribution of the shigella across different animal-based food samples.

SR NO.	SAMPLE	GENTAMYCIN	NALIDIXIC ACID	TRIMETHOPRIM	TETRACYCLINE	CIPROFLOXACIN	CHLORAMPHENICOL	ERYTHROMYCIN	AMPICILLIN	OFLOXACIN	CEFOPERAZONE
1	P4										
2	P8										
3	P10										
4	P11										
5	P12										
6	M18										
7	M19										
8	M20										
9	M21										
10	M22										
11	M23										
12	MT2										
13	MT3										
14	MT4										
15	MT5										
16	MT6										
17	MT7										
18	MT17										
19	MT19										
20	MT20										
21	MT21										
22	MT32										
23	C22										
24	C23										
25	C17										
26	F22										
27	F27										
28	F34										
29	F41										
30	F50										

Figure 2 Antibiotic susceptibility testing of the isolates against various antibiotics, S (Sensitive)- *I (Intermediate)-* *, R (Resistance)-*

Table 3 Percentage Distribution of antibiotic resistance in all isolates

ANTIBIOTIC	RESISTANT
Gentamycin	0%
Nalidixic acid	23.33%
Trimethoprim	36.66%
Tetracycline	86.66%
Ciprofloxacin	0%
Chloramphenicol	0%
Erythromycin	6.66%

Ampicillin	83.33%
Ofloxacin	0%
Cefoperazone	10%

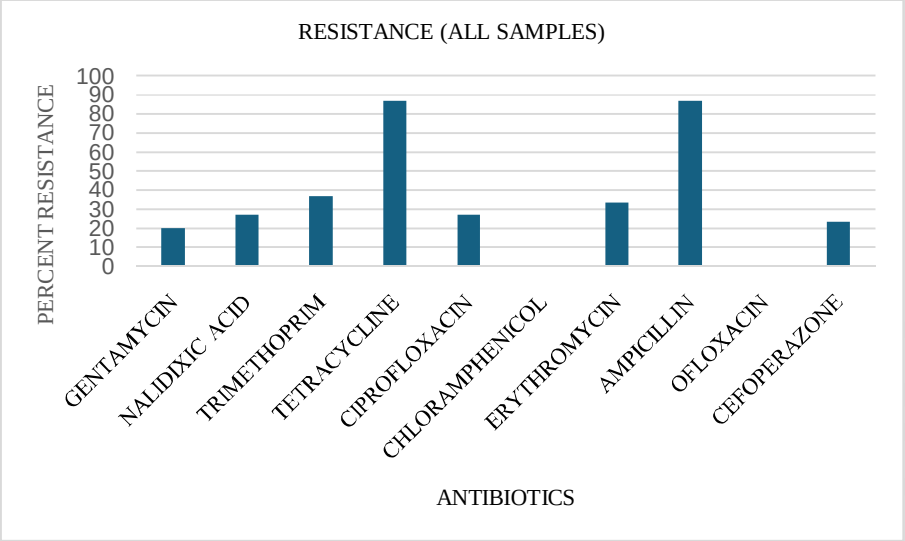

Figure 3 Graphical Distribution of antibiotic resistance in all isolates

Molecular characterization of the 30 Shigella isolates using 16S rna showed predominance prevalence of *Shigella dysenteriae*(53.3%) as shown in Table 2. While *S. flexneri* has traditionally been identified as the primary cause of shigellosis in developing nations, recent investigations suggest that *S. dysenteriae*, specifically serogroup A, is prevalent in Africa (Bintsis, 2017). This finding contrasts with the research by Ahmed and Shimamoto (2014), which found *Shigella spp.* in 1.4% of dairy product samples, with *S. flexneri* being the predominant species[1]. Therefore, it is imperative to implement fundamental hygienic practices in animal farms to mitigate the transmission of *Shigella* to both animals and humans. This includes controlling microbial contaminants, ensuring hygiene proficiency, maintaining the cleanliness of cows, managing animal health effectively, in effect cleaning and disinfection measures of the milking mechanism, providing clean water, and upholding stringent staff hygiene standards [9,13].

One of the paramount hurdles in ensuring food safety is the rise of antimicrobial-resistant bacterial strains. Resistance genes have the potential to be horizontally transmitted from animal food sources and environments to both the normal flora of humans and pathogens through the food and beverage supply chains [6]. The extensive use of a wide array of antibiotics in food animals, whether for disease prevention, veterinary treatment, or growth promotion, facilitates the emergence of multidrug-resistant (MDR) foodborne bacterial pathogens. Research carried out in Iran revealed that *Shigella spp.* isolated from clinical samples exhibited total resistance to chloramphenicol, ampicillin, tetracycline, streptomycin, and trimethoprim, with intermediate resistance observed for cefotaxime [12]. Furthermore, the current study indicated decreased susceptibility to CTX, CAZ (third-generation cephalosporins), and CIP, which are typically preferred medications for treating shigellosis[5,22]. Consequently, the emergence of such resistance presents a significant obstacle to effectively treating shigellosis.

In this study, the antibiotic sensitivity screening of all *Shigella* isolates involved nine antibiotics: Ciprofloxacin, Cefaparazone, Chloramphenicol, Erythromycin, Gentamycin, Nalidixic acid, Tetracycline, Trimethoprim, and Ofloxacin. This screening utilized the microbroth dilution technique, with the concentrations of antibiotics employed being predetermined. *Shigella* isolates recovered 86.66%, 83.33%, 36.66%, 23.33%, 10% and 6.66% were resistant to tetracycline, ampicillin, trimethoprim, nalidixic acid, cefoperazone and erythromycin respectively. One hundred percent were completely sensitive to gentamycin, ciprofloxacin, ofloxacin and chloramphenicol are shown in Table 3 and represented in Figure 3. These data indicate that there has been a considerable increase in resistance to different classes of antibiotics among the *Shigella* isolates.Mokhtari et al. also found significantly different antimicrobial susceptibility patterns of *Shigella* isolates obtained from food and clinical samples [15]. Despite fluoroquinolones being endorsed as the primary drugs for treating shigellosis by the World Health Organization (WHO 2005), the emergence of fluoroquinolone resistance among *Shigella spp.* has been reported in numerous countries [22,25].

It is important to highlight that the escalating trend of multidrug resistance observed in *Shigella* strains sourced from both food and clinical settings is recognized as a significant global issue and poses a serious threat to public health. Enforcing

rigorous hygiene protocols and implementing effective antibiotic management in food production and handling are essential to reduce the risk of foodborne infections and address the increasing threat of antibiotic resistance[11]. This knowledge will be valuable for ongoing monitoring and control efforts in meat and meat products, particularly in ready-to-eat foods, retail meat establishments, slaughterhouses, and fast-food restaurants [20].By focusing on prevention and mitigation strategies, we can effectively combat the presence of this significant pathogen within the food industry.

CONCLUSION

There was high prevalence of *Shigella* species isolated from different food samples and *S. dysenteriae* was the predominant species isolated from the samples. The emergence of resistance to many drugs such as nalidixic acid, erythromycin, trimethoprim, in *Shigella* is a cause of great concern. The study's focus on a specific geographic area may limit the applicability of its findings to other regions, potentially leading to either an underestimation or overestimation of *Shigella* prevalence. Furthermore, the lack of investigation into genetic determinants of antibiotic resistance in the isolated strains hampers our understanding of resistance mechanisms. Future research should prioritize molecular characterization of *Shigella* isolates to elucidate antibiotic resistance epidemiology. Establishing a network of laboratories to monitor *Shigella* antibiotic resistance in real-time and promptly sharing this information with clinicians for treatment strategy adjustments is urgently required to prevent and control shigellosis and alleviate its burden [23,19]. Overall, the study highlights the importance of monitoring and addressing multidrug-resistant *Shigella* in food samples to protect public health. The high levels of antibiotic resistance, particularly against commonly used drugs, underscore the need for enhanced surveillance and control measures.

Ethical statement

No animals were harmed during this study.

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ABBREVIATION

AMP-Ampicillin; GEN-Gentamycin; NA-Nalidixic acid; C-Chloramphenicol; CIP-Ciprofloxacin; CPZ-Cefaparazone; OF-Ofloxacin; E-Erythromycin; TR-Trimethoprim; TE-Tetracycline ;PCR-Polymerase Chain Reaction; XLD- Xylose-Lysine-Deoxycholate; PW-Peptone Water; SB-Selenite F Broth ; RVS-Rappaport Vassiliadis Soya; R-Resistant; S-Sensitive; I-Intermediate; MAR-Multiple Antibiotic Resistance; CLSI- Clinical and Laboratory Standards Institute; AST-Antibiotic Susceptibility Test; NCBI-National Center for Biotechnology Information; EIEC- Entero Invasive *Escherichia coli*