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The detection and antimicrobial susceptibility profile of *Shigella* isolates from meat and swab samples at butchers' shops in Gondar town, Northwest Ethiopia

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Summary Food borne pathogens are major causes of deaths, illnesses and billions of dollars of expenses. The burden of food borne illness is worsened by the ever increasing rate of antimicrobial resistance microbes. *Shigella*, a bacterial pathogen associated with food, is reported to account for higher prevalence rates of food borne illness in different settings. A cross-sectional study was conducted from February 10 to June 30, 2013, at the butcher houses of Gondar town in the Northwest of Ethiopia to assess the prevalence and antimicrobial susceptibility pattern of *Shigella*. Cattle raw meat and swab samples from selected critical control points, including knives, chopping boards, and the hands and noses of butchers, were collected and analyzed. The identification of *Shigella* was carried out using colony characteristics, the Gram reaction, and biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby—Bauer disc diffusion method. The overall hygienic status of the butcher shops was also assessed using a checklist. An observational analysis revealed that the sanitary condition of the butcher shops and their premises was poor. Of 306 samples screened, 10.5% were positive for *Shigella*.

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Approximately 7.4% of meat samples and 10.2% of swab samples were contaminated with *Shigella*. Out of the total *Shigella* isolates, 90.6%, 46.9%, 18.8% and 9.4% were resistant to ampicillin, amoxicillin, ceftriaxone and tetracycline, respectively. A multidrug resistance pattern was recorded in 27.8% of the isolates. In conclusion, the safety of meat sold at Gondar butchers houses was poor. The identified *Shigella* isolates showed high levels of drug resistance and multidrug resistance patterns for commonly used antimicrobials in veterinary and human medicine. Practicing wise use of antimicrobials and strict sanitary interventions at different critical control points is strongly recommended, in addition to further in-depth studies to prevent unprecedented consequences from shigellosis.

Introduction

Food-borne pathogens are among the leading cause of death and illness in developing countries and cost billions of dollars in medical care and social costs [1]. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygienic practices are major contributing factors [2]. Contaminated raw meat is one of the main sources of food-borne illnesses and is a risk factor for the transmission of zoonotic infections [3—5]. Food safety and quality is becoming a matter of increasing concern and a serious threat to public health in many countries. Biological contaminants, largely bacteria, constitute the major cause of food borne diseases [6].

The World Health Organization (WHO) estimated that in developed countries, up to 30% of the populations suffer from food borne diseases, and resulting in up to 2 million deaths in developing countries per year [7]. Bacteria contaminate meat while it is being processed, packed, transported and consumed. The contamination could be due to the ill health of the animal or due to slaughtering and subsequent processes [8]. *Shigella* is a bacterial contaminant of food from different sources and can result in illness with a low infective dose [9]. Food borne outbreaks of *Shigella* are also common, especially with foods that are subjected to hand processing, such as meat in butcher shops; exposed to a limited heat treatment; or served raw to the consumer [10]. Raw food materials of doubtful quality; storing food at a temperature that would permit microbial growth; the use of water of questionable hygienic quality; using packaging materials that are not of food-grade quality; lack of facilities for proper waste disposal at vending sites; and the use of unclean utensils are possible reasons for bacterial contamination of meat in butcher shops [11,12].

Previous reports from different parts of Ethiopia indicated that *Shigella* is circulating in the Ethiopian population with increasing antimicrobial resistance over time [13—20]. Selling and consuming raw meat at butcher shops are practiced widely in different towns of Ethiopia. Gondar town is one of these towns where raw meat is sold at butcher houses. However, there is scarce information on the microbial quality and safety of raw meat that is sold at butcher houses, particularly in relation to *Shigella* contamination. To the best of the authors' knowledge, to date, there has only been one study carried out on meat from an abattoir regarding *Shigella* isolates and their antimicrobial resistance pattern in Ethiopia [21]. Hence, the aim of this study was to determine the prevalence and antimicrobial susceptibility profile of *Shigella* from raw meat and selected critical control points from butcher houses in Gondar town.

Materials and methods

Study design, period and area

A cross sectional study was conducted from February 10 to June 30, 2013, at Gondar town, Northwest Ethiopia. The town is located at an altitude of 1500—2200 m above sea level. The maximum and minimum temperatures of the area were 30.7 °C and 12.3 °C, respectively. The area receives a bimodal rainfall pattern with an annual precipitation rate of 1000 mm. According to the 2007 Ethiopian central statistics agency summary report, the total human population of the town was estimated to be 206,987 [22]. During the study period, there were 90 operational butcher houses.

Sample size

All of the 90 butcher houses operating in the town were included in the meat sample collection procedures. A total of 306 samples, of which 90 were raw cattle meat samples from meat that was displayed for sale at butcher shops, 54 were knife swab samples, and 54 were surface swab samples from chopping boards that had direct contact with meat, were collected for bacteriological study. Meat samples were taken once from each butcher shop directly as it was sold to consumers. Swab samples from 54 noses and 54 hands of meat handlers were also collected. All of the swab samples were collected on a voluntary basis, but the meat samples were purchased from the butcher shops.

Data collection and laboratory analysis

Observational checklist

A checklist that was prepared for the purpose of this study was used to record observational findings related to the meat handling practices of the workers and their food safety knowledge as well as the general quality of the butcher shops, including their sanitary facilities and the conditions of the butcher houses and instruments.

Sample collection

Cattle raw meat samples and swab samples from selected critical control points that were considered to be associated with contamination were aseptically collected using separate sterile plastic bags for the meat samples and sterile test tubes for the swab samples. The swab samples were collected from 15 to 20 cm^2 of the surface of meat-cutting equipment (knives and wooden chopping boards) and the hands of meat handlers from each butcher shops.

Laboratory analysis

The identification of *Shigella* species

Twenty-five grams of meat were homogenized in 225 ml of 0.1% buffered peptone water (Oxoid, England) by shaking for 5 min in a sterile stomacher bag and incubating at 37 °C for 24 h for resuscitation. Swab samples were also incubated in 5 ml of buffered peptone water at a temperature of 37 °C for 24 h for enrichment. A loopful of the enriched samples was directly inoculated onto MacConkey agar (Oxoid, England) and *Salmonella—Shigella* agar (Oxoid, England) and was incubated at 37 °C for 24—48 h following previously described protocols [23—25].

Presumptive *Shigella* colonies were identified using a series of biochemical tests, including the triple sugar iron (TSI), lysine iron agar (LIA), urea, motility, citrate utilization, oxidase, indole production, methyl red (MR), Voges-Proskauer (VP), and recommended sugar tests [23,24]. *Shigella flexneri* ATCC 12022 was used as a quality control reference strain.

The antimicrobial susceptibility test

The antimicrobial susceptibility test of the isolates was performed according to the Clinical and Laboratory Institute (CLSI) and Bauer and his colleagues' method on Mueller-Hinton agar (Oxoid, England) [26,27]. *Escherichia coli* ATCC 25922 was used as a quality control organism for the antimicrobial susceptibility test. The antibiotics tested include amoxicillin (AML 2 μg), ampicillin (AMP 10 μg), tetracycline (TE 30 μg), gentamicin (CN 10 μg), trimethoprim—sulfamethoxazole (cotrimoxazole) (SXT 25 μg), ceftriaxone (CRO 30 μg), nitrofurantoin (F 100 μg), and nalidixic acid (NA 30 μg). All antibiotic discs were purchased from Oxoid Ltd. Company, Basingstoke, England. The selection of antimicrobials was based on the availability and frequency of use of these antimicrobials in the study area, both in veterinary and human medicine.

Data analysis and interpretation

The data were entered twice into a Microsoft Excel spreadsheet. Descriptive statistics were used to describe the frequency of *Shigella* species that were isolated from different samples. The results were summarized as percentages and are presented in tables and graphs.

Ethical considerations

The study was approved by the ethical review committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Official written permission and informed consent was obtained from concerned higher officials and study participants, respectively. All of the information obtained from the study participants was kept confidential.

Results

General quality and hygienic status of the butcher houses and meat handlers

The majority of the butcher houses had wooden and mud walls, roofs made of corrugated sheets of iron

Table 1 General conditions of butcher shops in Gondar town, Northwest Ethiopia.

Checkpoints		Frequency	Percentage
Butcher shop floor is constructed of:	Concrete	23	25.6
	Tile	3	3.3
	Earthen material	60	66.7
	Wood	4	4.4
The floor of butcher shop is free of large cracks		41	45.6
Butcher shop has a ceiling		61	67.8
Wall and ceiling free of dust and spider webs		33	36.7
Wall of butcher shop is painted with white paint		49	54.4
Presence of a shelf to display meant		66	73.3
Presence of insect-proof shelf		43	47.8
Availability of a functional refrigerator		27	30
Chopping board is made up of:	Wooden material	84	93.3
	Concrete	1	1.1
	Marble	4	4.4
	Plastic	1	1.1
Chopping board is smooth & easily washable		30	33.3
Presence of a clean meat hanger		25	27.8
Knives & other equipment handled in a sanitary condition		25	27.8
Materials used to wrap-up meat:	Appropriate paper	0	0
	Plastic festal	32	35.6
	News paper	33	36.7
	Used paper	24	26.7
Personal hygiene: Use of clean clothing (especially gowns) and clean hands		6	6.7
Presence of a hand washing basin at the front of the shop		0	0
Availability of first aid kits		0	0
Personnel have training and a certificate		0	0
Habit of handling money with bare hands		90	100

without ceilings and muddy floors. The walls were painted with white ink and supplied with 60—120 V electric light. Observational analysis of the current research revealed that the majority ($n = 60$, 66.7%) of the butcher shop floors were constructed from earthen materials and 54.4% had cracks of different sizes. Sixty-three (70%) butcher shops had no refrigerator for meat preservation, and 84 (93.3%) of the butcher shops had chopping boards made of wooden materials, which were not smooth or easily washable. None of the butcher shops used appropriate types of material to wrap up meat and none had a first aid kit. Sixty-five (72.2%) of the butcher shop meat hangers were not clean (they were observed to be dirty), and 65 (72.2%) of the butcher shops' knives and other equipment were not handled in sanitary ways. Moreover, only 43 (47.8%) of the shelves used to display the meat were insect proof, i.e., had appropriate preventive mechanisms installed to protect the meat from rodents and insects. None of the butchers used gloves or received training on how to handle meat, as presented in Table 1.

The prevalence of *Shigella* isolates and their antimicrobial susceptibility profile

From the total samples analyzed, *Shigella* was isolated from 32 samples, giving an overall identification rate of 10.45%. Ten meat samples (7.4%), 7 hand swabs (10.9%), 7 knife swabs (10.9%) and 8 chopping board swabs (12.5%) were positive for *Shigella* species. None of the nasal swab samples were positive for *Shigella*.

The antimicrobial susceptibility profile of the isolates revealed a higher rate of resistance against ampicillin (90.6%) and amoxicillin (46.9%). On the other hand, all of the *Shigella* isolates were susceptible to gentamicin and trimethoprim—sulfamethoxazole (cotrimoxazole). In addition, 90.6%, 81.2%%, 71.9% and 68.8% of the isolates were susceptible to tetracycline, nalidixic acid, nitrofurantoin and ceftriaxone, respectively, as shown in Fig. 1. The antimicrobial resistance patterns of the *Shigella* isolates were random throughout the samples and butcher shops.

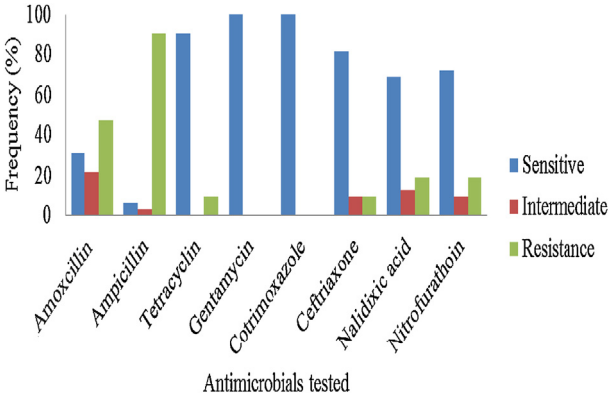

Figure 1 Antimicrobial susceptibility pattern of Shigella isolates.

All of the isolates identified from meat samples and chopping board swabs were resistant to ampicillin. In contrast, all of the isolates harvested from knife swabs were susceptible to cotrimoxazole and gentamicin. The resistance pattern was random throughout the town. Eighteen (56.3%) of the *Shigella* isolates subjected to the antimicrobial susceptibility test exhibited resistance to two or more antimicrobial agents, as shown in Table 2. Among the isolates that demonstrated resistance to more than one antimicrobial, 3 (16.7%), 4 (22.2%), 5 (27.8%) and 6 (33.3%) were isolated from hand swabs, chopping board swabs, knife swabs and meat samples, respectively. Multidrug resistance was recorded in more than one-quarter of the isolates. The highest resistance pattern isolates were identified from meat samples, followed by knife swab samples. Information gathered from different sources on the use of antimicrobials as growth promoters or feed additives in animals indicated that there is no such practice in the research area to date, unlike in developed countries.

Discussion

In the present study, approximately 72.2% of the butcher shop knives and other equipment were not handled in a sanitary condition. In more than half of the butcher shops, the shelves used to display meat were not insect proof. Approximately 70% of the shops had no refrigerator for meat preservation. None of the butcher shops used an appropriate type of paper to wrap meat, and none of the butchers used gloves. This situation may be attributable to the low level of knowledge of the butchers on how to handle meat, which is evidenced by the fact that none of them had training in food handling. All of the good food handling indicators assessed were against safe food handling requirements, which include cleaning the equipment and premises, personal hygiene, temperature control and the prevention of cross contamination [28]. A lack of sanitary conditions is the most common cause of contamination of meat from different sources, including money, as we found similar organisms both on the meat and the sources of contamination [29].

The WHO—FAO joint document on food hygiene stated that the following conditions need to be satisfied for the safety and suitability of food: the surfaces of the floors and walls should be made of impervious materials with no toxic effect in intended use; the floors should be constructed to allow adequate drainage and cleaning; the walls should have a smooth surface up to a height appropriate for the operation; and the ceilings and overhead fixtures should be constructed and finished to minimize the build-up of dirt and condensation and the shedding of particles [30]. Despite these facts, the butcher shops included in this report did not satisfy most of the preconditions established in this document.

Table 2 Multiple antimicrobial resistance profile of *Shigella* isolates.

Resistance for no. of antimicrobial	Antimicrobials (no. of isolates)	Resistance category	Total no. of isolates (%)
Two	AMP, AML (5)	Drug resistant	6 (33.33)
	AMP, NA (1)	Drug resistant	
Three	AMP, AML, F (2)	Drug resistant	10 (55.55)
	AMP, NA, F (2)	Multidrug resistant	
	AMP, CRO, F (1)	Multidrug resistant	
	AMP, AML, TE (1)	Drug resistant	
	AMP, AML, CRO (4)	Drug resistant	
Four	AMP, AML, TE, F (1)	Multidrug resistant	2 (11.11)
	AMP, TE, NA, CRO (1)	Multidrug resistant	

Key: AML = amoxicillin, AMP = ampicillin, TE = tetracycline, CN = gentamicin, SXT = trimethoprim—sulfamethoxazole (cotrimoxazole), NA = nalidixic acid, CRO = ceftriaxone, F = nitrofurantoin.

The 10.5% isolation rate of *Shigella* species in the current study was lower than a study conducted in Pakistan, which detected a prevalence of 12.9%. The 10.2% *Shigella* prevalence obtained from the swabs in our study was higher than the Pakistani study, which reported an overall isolation rate of 4.4% [5]. This might be due to the poor hygienic status and improper equipment handling in the butcher shops in the current study area. On the other hand, this study reported a very high isolation rate of *Shigella* species compared to a study conducted in Jimma town, Ethiopia, which reported a prevalence of 0.6% among samples taken from different settings [12]. This difference may be ascribed to the smaller sample size included in the study under comparison. A similarly lower rate of isolation was reported from Ebony state of Nigeria [31]. When compared to studies conducted in abattoirs, the prevalence of *Shigella* species in our study was still higher. Previous studies in Ethiopia [21] and Nigeria [32] abattoirs found no *Shigella* species. In general, the higher rate of contamination of meat and equipment in the butcher shops is an indication of the poor hygienic and sanitary practices exercised during the slaughtering, transportation, and/or processing of the meat [9].

The antimicrobial resistance patterns of *Shigella* isolates identified during the current study were random among the samples and butcher shops. The *Shigella* isolates cultivated in this study were highly resistant (90.6%) to ampicillin, which is consistent with studies in Karachi, Pakistan [5], and Eastern Nepal [33], which reported resistance rates of 72% and 86.8%, respectively. The present study also revealed a multidrug resistance pattern in 27.8% of the isolates according to the recent interim standard dentitions for acquired resistance [34]. The multidrug resistance pattern recorded in the current research might be due to the emergence of antibiotic resistant strains of *Shigella* due to evolution or antibiotic pressure from the unrestricted use of antibiotics by the community and animal health professionals. In addition to the above reports, a five-year survey by the Drug Administration and Control Authority of Ethiopia reported results comparable with our finding [35].

In this investigation, tetracycline was found to be among the most effective drugs next to gentamicin, trimethoprim—sulfamethoxazole and ceftriaxone against the *Shigella* isolates. Tetracycline has been among the most frequently prescribed drugs both in human and veterinary medicines for more than three decades in the current study area. In contrast, *Shigella* isolates that were 100% and 89.5% resistant to tetracycline were reported by Ali and his colleagues from Pakistan [5] and the drug administration and control authority of Ethiopia [35], respectively. Differences in the geographical location of the isolates or the emergence of drug resistant strains of *Shigella* could partially explain this discrepancy.

Conclusion

The high prevalence of *Shigella* species isolated from fresh meats and other critical control points in this study might be attributed to poor personnel hygiene and the low sanitary standards of the butcher houses and their premises in the current study area. Such a high prevalence is a warning signal for the possible occurrence of food borne illness and potential food borne outbreaks. The present investigation revealed the circulation of moderate to high degrees of drug resistant and multidrug resistant *Shigella* isolates in the study area for antibiotics in frequent use for human diseases treatment in Ethiopia. Cheap and readily available antibiotics, such as amoxicillin and ampicillin, are not effective against these isolates. The current study highlights the need for the further investigation of the prevalence and antimicrobial susceptibility pattern of *Shigella* from the farm to the table to trace contamination sources. The characterization of resistant strains at the molecular level and identification of resistance mechanisms are highly recommended. Judicious and prudent use of antimicrobials in both the veterinary and public health sectors should be mandatory because high rates of antimicrobial resistant isolates were identified.

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Competing interests

None declared.

Ethical approval

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