ISSN: 3033-3504

Journal of Clinical Medicine and Regenerative Medicine

SCIENTIFIC Research and Community

Research Article

Open Access

One-Health Approach to Determine and Tackle Antimicrobial Resistance in the Human Dairy Interface: A Case of Non-Typhoidal Salmonella and Lactose-Fermenting Enterobacteriaceae

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ABSTRACT

Background

Antimicrobial resistance (AMR) is among the top public health concerns globally. Determining the susceptibility pattern of pathogens is important in designing strategies to combat AMR. Thus, this study was designed to determine the AMR pattern of non-typhoidal Salmonella (NTS) and lactose fermenting Enterobacteriaceae (LFE) isolated from the human-dairy interface in the northwestern part of Ethiopia, where such information is lacking.

Methods

The study analysed 362 samples collected from humans, animals, food (milk) and the environment (sewage). The bacteria were isolated from the samples using standardized bacteriological methods. The antimicrobial susceptibility patterns and extended-spectrum beta-lactamase (ESBL) production ability were screened and confirmed by using the Kirby-Bauer disk diffusion method. The isolates were further characterized genotypically using multiplex polymerase chain reaction targeting the three ESBL-encoding genes.

Results

A total of 28 and 375 NTS and LFE bacterial isolates were identified. Isolates were more resistant to ampicillin and tetracycline. Forty-six point four and 70.7% of NTS and LFE were multidrug resistant (MDR), respectively. None of NTS and 21.3% of LFE were ESBL-producing. Genotypically, the majority of the isolates (97.5%), which were positive on the phenotypic test, were carrying one or more ESBL encoding genes. In conclusion, a high proportion of the bacterial isolates were resistant to commonly used antimicrobials, MDR, and were positive for ESBL production.

Conclusion

The findings provide evidence that the human-dairy interface is one of the important reservoirs of AMR traits and intervention points to reduce AMR. Therefore, the implementation of AMR mitigation strategies in a one-health approach is highly needed in the area.

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Received: July 14, 2025; **Accepted:** July 25, 2025, **Published:** July 28, 2025

Keywords: Antimicrobial Susceptibility, Extended-Spectrum Beta-Lactamase, Lactose Fermenting Enterobacteriaceae, Human-Dairy Interface, Multidrug-Resistance, Non-Typhoidal Salmonella, Northwest Ethiopia

Methodology

Study Area

The study was conducted in northwest Ethiopia from June 2022 to August 2023. For the survey, two cities (Bahir Dar and Gondar), seven districts (Dangila, Fogera, Kemkem, Takusa, Bahir Dar Zuria, Gondar Zuria, and Debark) were selected based on their relatively higher dairy production activities (Figure 1). Milk production in the areas is dominated by small-scale farms with mixed farming systems. However, intensive or semi-intensive dairy farms are also available, particularly in and around urban areas.

Figure 1: Map of the Sampling Sites

Study Populations

The targeted population for this study were dairy farms in Northwest Ethiopia. To select the dairy farms first, a list of dairy farmers was obtained from livestock development offices in all selected sites. The dairy farms to collect samples were selected randomly by the lottery method. In the selected farms, the pooled raw milk produced from the farm, milk containers, contact persons (milkers), and the environment (dairy farm sewage) were sampled. All humans and animals sampled were healthy and were not taking antimicrobials at the time of sampling. All animals were lactating cows.

Sample Collection and Bacteria Identification

Approximately 50 ml of pooled raw milk was collected aseptically. Swabs from milkers' hands and milk containers were collected separately by using sterile cotton swabs and placed into test tubes containing sterile buffered peptone water (BPW) (Garnaut, Germany). About 10 ml of effluent samples were collected and placed into sterile containers. Similarly, about 10 grams of stool samples were collected in sterile stool cups with applicator sticks from milkers. Finally, approximately 10 grams of faecal samples were collected directly from the rectum of randomly selected lactating dairy cows using sterile disposable gloves into the sterile screw-capped tube. Samples were transported using a cool box (~4oC) to the food safety laboratory of the University of Gondar, stored at ~4oC) and processed within 24 hours of collection.

Isolation of Non-Typhoidal Salmonella

The isolation of NTS was performed using the techniques recommended by the International Organization for Standardization [6]. Each sample type was first enriched non-selectively in BPW at a 1:10 ratio. Swab samples transported in 9 ml of BPW were incubated directly. Three grams of cow's faeces, human stool, and sewage samples were separately transferred into a sterile container with 27 ml of sterile BPW. Twenty-five ml of the milk sample was transferred into a sterile screw-caped glass bottle containing sterile 225 ml BPW. The sample with enrichment fluid was mixed thoroughly and incubated at 37°C for 24 hours.

The Rappaport-Vassiliadis soya (RVS) broth (Oxoid, England) and Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth (Oxoid, UK) were used for selective enrichment. From the enriched fluid, 0.1 ml was transferred into a tube containing 10 ml of RVS broth and incubated at 41.5°C for 24 hours. Another 1 ml of the enriched sample was transferred into a tube containing 10 ml of MKTTn broth and incubated at 37°C for 24 hours. Then, a loopful of enriched samples was inoculated on xylose lysine deoxycholate agar (XLD) (Hi-Media, India) and Hektoen enteric (HE) agar (Thermofisher, Netherlands). After incubation, the plates were examined for the presence of typical and suspected colonies (pink colonies with or without black centres on XLD agar and blue-green to blue colonies with or without black centres on HE agar) [6,7]. From both selective plating mediums, three to five suspected colonies were taken and streaked separately over the surface of tryptone soya agar (Sigma-Aldrich, USA), and then incubated for 24 hours at 37°C for biochemical characterization. All presumptive Salmonella isolates that fulfilled the preliminary biochemical tests were further characterized by the analytical profile index (API) 20E test (BioMerieux Inc., France). All Salmonella isolates that passed the API 20E test were confirmed by Matrix-assisted Laser Desorption Ionization–Time-of-Flight mass spectrometry (MALDI-TOF-MS, Bruker, USA).

Isolation of Lactose-Fermenting Enterobacteriaceae

Escherichia coli and other LFEs were detected and isolated as described in the FDA Bacteriological Analytical Manual ISO protocols [8-10]. With slight modifications, as follows. The sample was homogenized and enriched in coliform selective broth (Oxoid Ltd, UK) at a 1:10 ratio (25 ml milk mixed with 225 ml broth, or 3 g of sewage, faeces, or stool mixed with 27 ml broth). Swabs in buffer peptone water were incubated directly. The sample-containing broth was incubated at 37oC for five hours and 42 oC for about 36 hours, and a loopful of the enriched sample was transferred to MacConkey agar (Oxoid Ltd) and incubated at 37 °C for 24 hours. Three to five well-isolated and lactose fermenting colonies were subcultured into Levine Eosin Methylene Blue Agar (Neogen Culture Media, UK) and incubated at 37 °C for 24 hours. After incubation, the bacteria were sub-cultured on tryptic soya agar (Sigma-Aldrich chemie GmbH, City, Germany) for biochemical tests. The colonies were further characterized by the API 20E (Biomerieux, France) test for identification [11].

Antimicrobial Susceptibility Test

The antimicrobial susceptibility test was performed using the Kirby-Bauer disk diffusion method [12]. Briefly, each bacterial isolate was harvested and deposited into a tube containing 0.85% sterile saline, vortex mixed, and the turbidity of the suspension was adjusted to match the 0.5%-McFarland standards. A sterile cotton swab was deposited into the suspension and then uniformly streaked over the entire surface of the Mueller-Hinton agar (MHA) (Sigma-Aldrich chemie GmbH, Germany). Paper discs impregnated with a fixed concentration of antimicrobials (Oxoid Ltd, UK) were placed on the

agar surface of inoculated agar, and plates were incubated at 37 OC for 24 hours. After incubation, the zone of inhibition was measured in millimetres using a calliper and interpreted as "susceptible", "intermediate" and "resistant" according to Clinical and Laboratory Standards Institute (CLSI) criteria [13]. For NTS, the isolate was tested for a set of 13 antimicrobials, which are grouped into 8 classes, whereas for LFE sixteen AMAs in nine antimicrobial classes were used to assess the antimicrobial susceptibility pattern. The bacterial isolates were grouped as MDR if they were resistant to at least one AMA in three or more classes [14,15].

Detection of Extended-Spectrum Beta-Lactamase-Production

To detect ESBL production, the isolates were screened against ceftriaxone, cefotaxime, and ceftazidime using the Kirby-Bauer disk diffusion method and interpreted based on the CLSI guideline [13]. Bacterial isolates exhibiting a zone of inhibition equal to or less than 22 mm for ceftazidime, 25 mm for ceftriaxone, or 27 mm for cefotaxime were preliminarily identified as potential ESBL producers. These isolates were then marked for subsequent phenotypic confirmation. Phenotypic confirmation was conducted using a combined-disk diffusion test. An isolate that passed the screening test was emulsified in 0.85% sterile saline solution, and its turbidity was matched with 0.5 McFarland standards and then inoculated on MHA by using sterile swabs. Cefotaxime (30 µg), and cefotaxime/clavulanic acid (30µg/10µg) disks were applied on the plate separately with at least 25 mm space between them, and then plates were incubated at 37 OC for 24 hours. If there is a greater than or equal to 5 mm increase in inhibition zone diameter for cefotaxime in combination with clavulanic acid as compared with the zone diameter of the tested cefotaxime alone, was considered an ESBL-producing isolate [13]. The genotypic confirmation was conducted by using multiplex PCR targeting the three ESBL-producing genes, namely blaTEM, blaSHV, and blaCTX-M [16].

The DNA was extracted from each phenotypically confirmed ESBL-producing isolate by heat lysis [17,18]. Amplification of target genes was carried out in a total volume of 20 µl reaction containing 10 µl of Master mix (hot start GoTaq green Master mix, Promega USA), 0.75 µl of 10 µM each primer (Table 4), 3.5 µl nuclease-free water and 2 µl template DNA. The genes were amplified in a thermocycler machine (MasterCycler, China) which was adjusted at initial denaturation of 95oC for 15 minutes, with 30 cycles consisting of denaturation at 95 oC for 60 seconds, annealing 60oC for 40 seconds, and extension at 72oC for 60 seconds and then followed by a final extension at 72oC for 5 minutes. The amplified products were visualized after gel-electrophoresis using 1% agarose gel after staining with ethidium bromide. A 100-bp DNA ladder (Quick-Load ® Purple DNA Ladder; Biolabs, England) was used as a molecular size marker to estimate the size of the PCR products. Amplicons were run at 140 V for 50 minutes and visualized under an ultraviolet transilluminator, then the image was taken using the gel documentation system (BioTop, Gel documentation system, China).

Data Quality

Maximum effort was made to keep the quality of the data starting from collection, storage, and analysis. All bacteriological media were prepared based on the manufacturer's instructions. The sterility of the media and other preparations was checked as frequently as possible. Known standards were used in the characterization of bacterial species. Escherichia coli, Klebsiella pneumoniae, and Salmonella enterica strains were used as quality control during the running of each laboratory test.

Results

Non-Typhoidal Salmonella and Lactose-Fermenting Enterobacteriaceae in the Human-Dairy Interface

The overall proportion of NTS was 7.7% with a 95% confidence interval of 5.4 - 11.0 %. The highest proportion of NTS was observed in cows' faeces (11.9%), followed by dairy farm sewage (10.5%), whereas the lowest was in the milker's hand swab samples (1.7%) (Table 2). Table 2 also depicts the proportion of NTS in different sampling sites. The highest proportion was on dairy farms found in Takusa (14.3%), followed by Fogera (11.1%), and the lowest was among samples collected in Dangila districts (4.5%).

Table 1: The Proportion of Non-Typhoidal Salmonella Isolates Based on Sample Types and Sampling Sites

Character of the Samples	Categories	Number examined	Number positive	Percent
Sample type	Cow's faeces	84	10	11.9
	Dairy farm sewage	57	6	10.5
	pooled Raw milk	58	6	10.3
	Milk container swabs	58	3	5.2
	Milker's stool	47	2	4.3
	Milker's hand swabs	58	1	1.7
Sampling sites	Takusa	35	5	14.3
	Fogera	36	4	11.1
	Bahir Dar	57	6	10.5
	Gondar	44	3	6.8
	Gondar Zuria	37	2	5.4
	Debark	38	2	5.3
	Kemkem	36	2	5.6
	Dangila	44	2	4.5
	Bahir Dar Zuria	35	2	5.7
Total		362	28	7.7

Among samples examined (n=362), 331 (91.4%) were positive for either of the LFEs. Escherichia coli (71.3%) was the dominant isolate in all samples, followed by Citrobacter spp (15.2%), Enterobacter spp (10.2%) and Klebsiella spp (6.9 %) (Table 3).

Table 2: The Proportion of Lactose-Fermenting Enterobacteriaceae in Different Samples and Sampling Sites

Factors	Categories	No tested	Overall LFE (n (%))*	E. coli (n (%))	Citrobacter spp (n (%))	Enterobacter spp (n(%))	Klebsiella spp (n(%))
Sample type	Raw milk	58	54 (93.1)	32 (55.2)	13 (22.4)	12 (20.7)	7 (12.1)
	Milk container swabs	58	52 (89.7)	30 (51.7)	17 (29.3)	10 (17.2)	7 (12.1)
	Milker's hand swabs	58	46 (79.3)	26 (44.8)	10 (17.2)	10 (17.2)	7 (12.1)
	Farm sewage	57	52 (91.2)	51 (89.5)	2 (3.5)	0 (0.0)	1 (1.8)
	Milker's stool	47	47 (100.0)	42 (89.4)	4 (8.5)	2 (4.3)	2 (4.3)
	Cow's faeces	84	80 (95.2)	77 (91.7)	9 (10.7)	3 (3.6)	1 (1.2)
Sampling sites	Gondar	44	38 (86.4)	30 (68.2)	8 (18.2)	3 (6.8)	0 (0.0)
	Bahir dar	57	51 (89.5)	47 (82.5)	4 (7.0)	7 (12.3)	1 (1.8)
	Dangila	44	39 (88.6)	29 (65.9)	7 (15.9)	4 (9.1)	4 (9.1)
	Fogera	36	35 (97.2)	28 (77.8)	2 (5.6)	5 (13.9)	3 (8.3)
	Kemkem	36	35 (97.2)	25 (69.4)	8 (22.2)	6 (16.7)	1 (2.8)
	Gondar Zuria	37	31 (83.8)	22 (59.5)	5 (13.5)	4 (10.8)	5 (13.5)
	Takusa	35	34 (97.1)	26 (74.3)	6 (17.1)	5 (14.3)	3 (8.6)
	Bahir dar Zuria	35	33 (94.3)	25 (71.4)	4 (11.4)	2 (5.7)	6 (17.1)
	Debark	38	35 (92.1)	26 (68.4)	11 (28.9)	1 (2.6)	2 (5.3)
Total		362	331 (91.4)	258 (71.3)	55 (15.2)	37 (10.2)	25 (6.9)

One sample may contain more than one LFE, hence, if individual bacteria may not be equal to the overall LFE; LFE = lactose fermenting Enterobacteriaceae, No or n = number, % = percent

Antimicrobial Susceptibility Profile

Non-typhoidal Salmonella isolates (n = 28) were tested against 13 commonly used antimicrobials representing 8 classes. The result showed that a higher proportion of drug resistance was observed in ampicillin (57.1%), tetracycline (42.9%) and chloramphenicol (35.7%). On the other hand, NTS isolates were more susceptible to ceftriaxone (100%), norfloxacin (96.4%), and azithromycin (96.4%) (Figure 2). Among the NTS isolates tested, 89.3% were resistant to one or more classes and 46.4% were resistant to more than two classes of antimicrobial agents (multi-drug resistance (MDR).

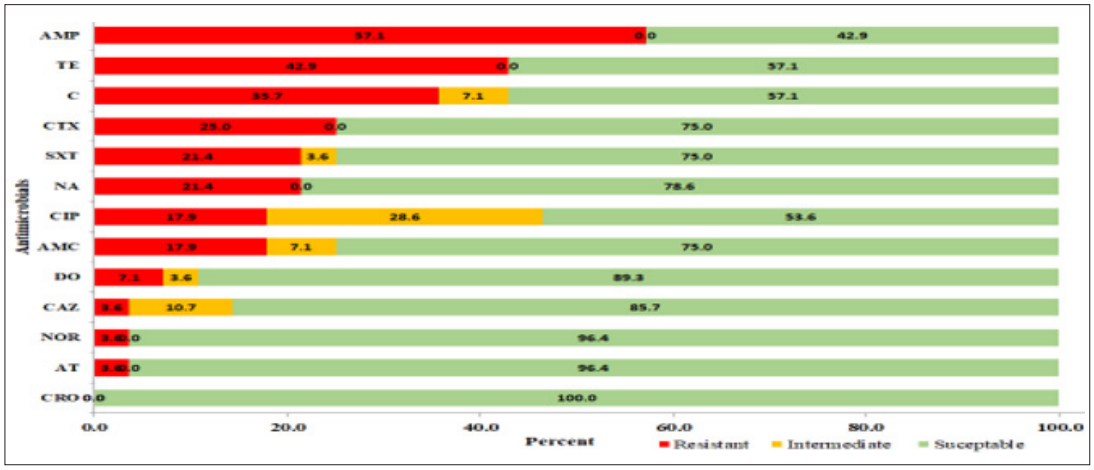

Figure 2: Antimicrobial Susceptibility Patterns of 28 Salmonella Isolates.

AMP=ampicillin; AMC=amoxicillin-clavulanic acid; CRO=ceftriaxone; CTX=cefotaxime; CAZ=ceftazidime; C=chloramphenicol; TE=tetracycline; DO=doxycycline; AT=azithromycin; NA=nalidixic acid; NOR=norfloxacin; CIP=ciprofloxacin; SXT= sulphamethoxazole-trimethoprim. (Isolates were divided into susceptible, intermediate, or resistant based on CLSI guidelines [13]. A greater proportion of the isolates were resistant to AMP, and all were susceptible to CRO.

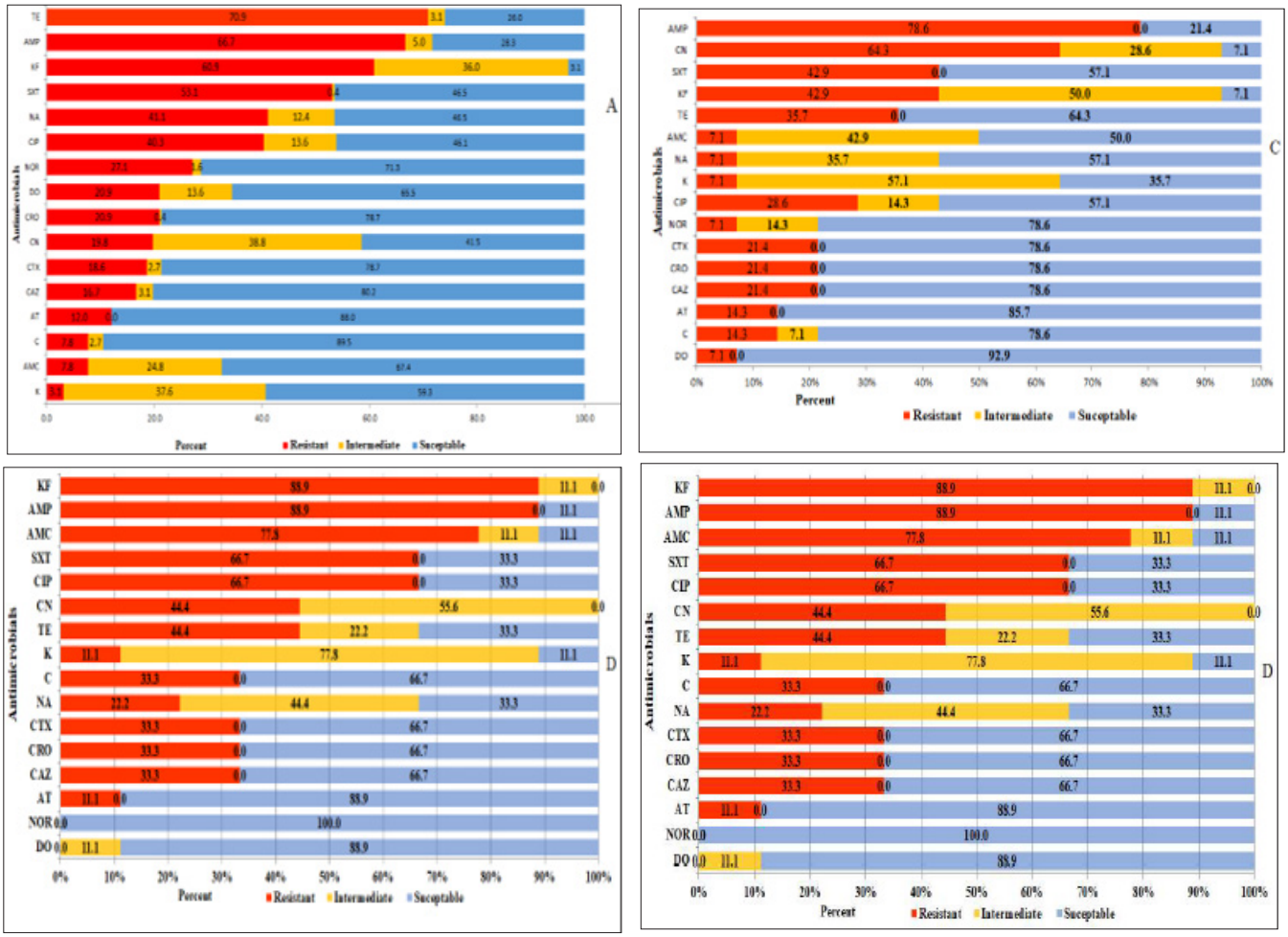

Figure 3: Antimicrobial Susceptibility Patterns Lactose Fermenting Enterobacteriaceae Isolates

(A= E. coli (N=258), B= Citrobacter spp (N=55), C= Klebsiella spp (N=25), D=Enterobacter spp (N=37), AMP= Ampicillin, AMC= Amoxicillin-clavulanic acid, KF= Cephalothin, CRO= Ceftriaxone, CTX= Cefotaxime, CAZ =Ceftazidime, C=chloramphenicol, TE=Tetracycline, Do=Doxycycline, AT= Azithromycin, CN=Gentamicin, K=Kanamycin, NA= Nalidixic acid, NOR= Norfloxacin, CIP= Ciprofloxacin SXT= (Sulphamethoxazole-trimethoprim) (Bacterial isolates were divided as "susceptible", "intermediate" or "resistant" based on CLSI guideline [13].

Multi-Drug Resistance Patterns

Among the bacterial isolates, 70.7% were MDR. Based on the type of bacteria, 73.0%, 72.5%, 65.5%, 60.0% and 46.4% of isolates of Enterobacter spp., E. coli, Citrobacter spp., Klebsiella spp., and NTS were MDR, respectively.

Extended-Spectrum Beta-Lactamase Production among Isolates

Phenotypic Characterization

The overall proportion of ESBL production was 80/375 (21.3%). Figure 20 depicts the screening and phenotypic confirmation of ESBL-producing bacterial isolates. Extended-spectrum β-lactamase (ESBL) production was detected in all types of bacterial isolates that belong to lactose-fermenting Enterobacteriaceae, and none of the NTS were producers. There was no statistically significant difference among bacterial isolates and the type of samples. However, the proportion was the highest among Enterobacter spp. (29.7%) and isolates from pooled raw milk (31.7%). Among sampling sites isolates from Bahir Dar (n=58) (29.3%) and Gondar (n=42) (28.6%) showed more ESBL detection than other sampling sites, and none of the isolates from Takusa were positive (n=44) for ESBL (Figure 4).

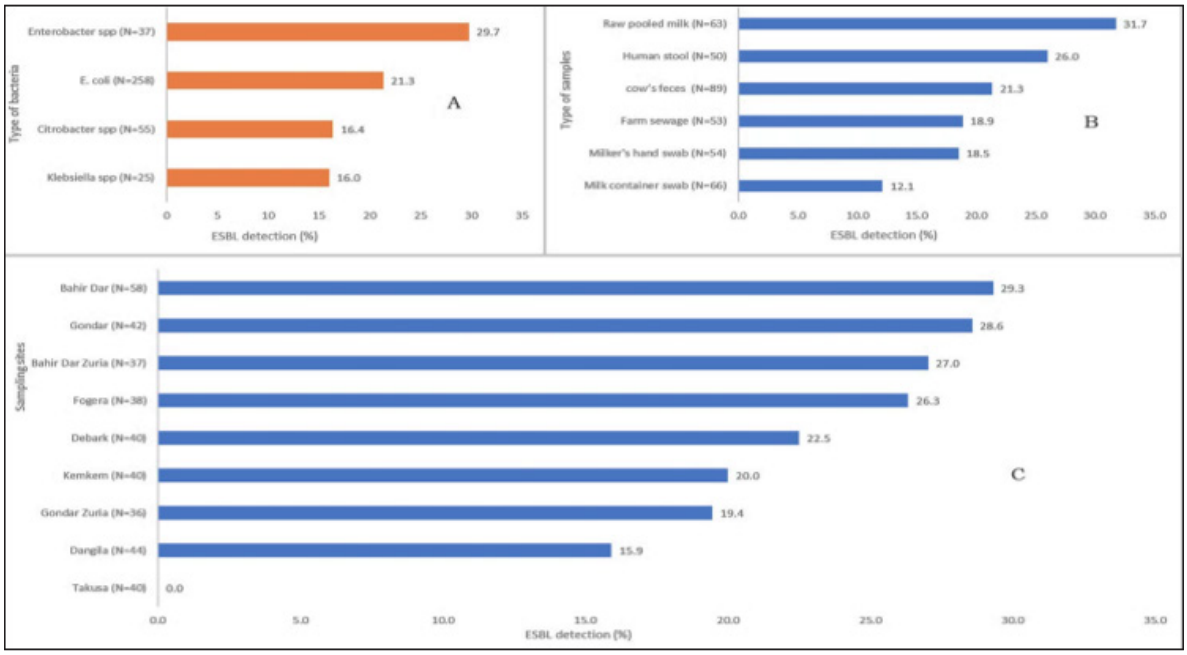

Figure 4: The Proportion of Extended-Spectrum Beta-Lactamase-Producing Lactose Fermenting Enterobacteriaceae (Lfe) As Per the Type of Bacterial Isolates (A), type of samples (B), and sampling sites (C), N=number of isolates tested, %= percent

All ESBL-producing bacterial isolates were MDR. Figure 5 shows the comparison of the percent of resistant isolates among ESBL non-producing and producing LFE. It was observed that ESBL producers tend to be more resistant to the majority of antimicrobials than non-producers except doxycycline. The ESBL producers were highly resistant to cephalothin (98.8%), ampicillin (98.8), ceftriaxone (95.0%), sulphamethoxazole - trimethoprim (92.5%) and cefotaxime (88.8%) (Figure 5).

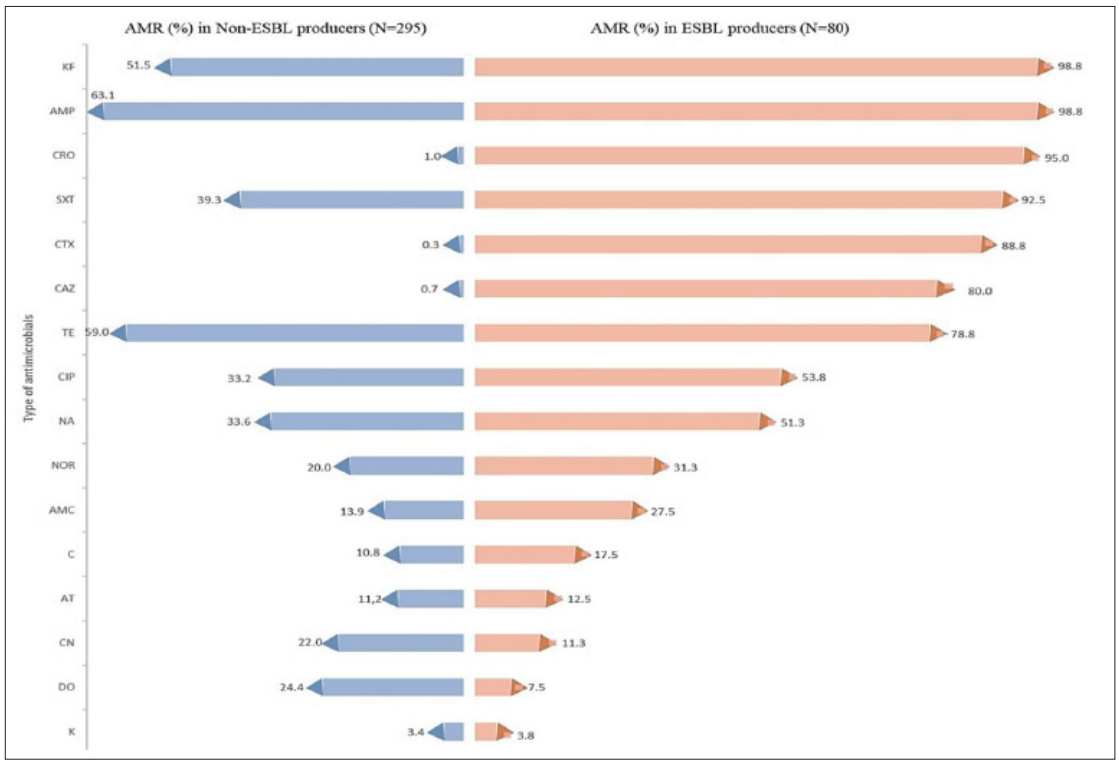

Figure 5: Comparison of the Antimicrobial Resistance (Amr) Proportion among Extended-Spectrum Beta-Lactamase (Esbl) Non-Producers and Producers (AMP=Ampicillin; AMC=Amoxicillin-clavulanic acid; KF=Cephalothin; CRO=Ceftriaxone; CTX=Cefotaxime; CAZ=Ceftazidime; C=Chloramphenicol; TE=Tetracycline; Do=Doxycycline; E=Erythromycin; AT=Azithromycin; CN=Gentamicin; K=Kanamycin; NA=Nalidixic acid; NOR=Norfloxacin; CIP=Ciprofloxacin; SXT=Sulphamethoxazole-trimethoprim) (In most cases, the proportion of resistant isolates was higher among ESBL producers than non-producers, except DO and CN)

Genotypic Characterization

The majority of the isolates (97.5%) were positive for the ESBL phenotypic confirmation test and were carrying at least one of the ESBL-encoding three target genes (blaTEM, blaSHV, and blaCTX-M). All three genes were detected among the bacterial isolates but blaCTX-M (85.0%) and blaTEM (78.8%) were the dominant (Figure 6). Figure 6 also depicts the distribution of the ESBL-encoding genes among phenotypically positive isolates, whereas figure 7 shows the representative PCR product used to detect the presence of target genes.

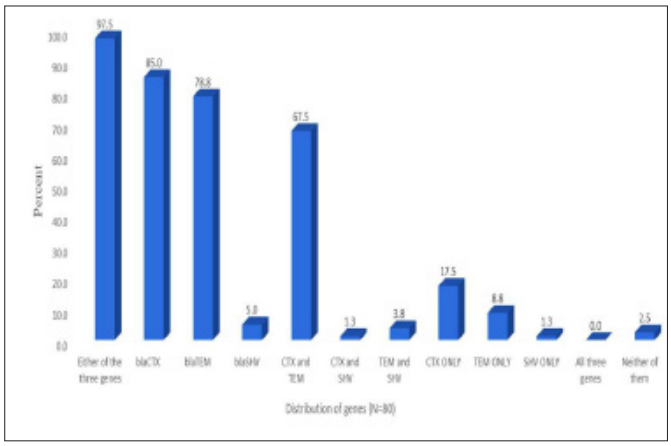

Figure 6: Distribution of Extended-Spectrum Beta-Lactamase (Esbl) Encoding Genes Among Phenotypically Esbl-Positive Isolates

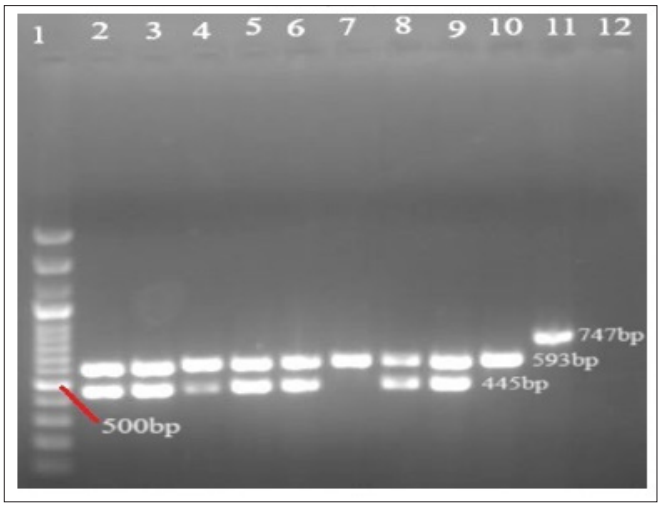

Figure 7: Representative Gel Picture (Pcr Products) in Gel Documentation (Lane 1 was ladder, lane 2,3,4,5, 6, 7, 8, 9, and 10 blaCTX-M positive at 593 base pair (bp); Lane 2, 3, 4, 5, 6, 8 and 9 were blaTEM positive at 445bp; Lane 11 was positive control which as positive only for blashv gene at 747bp, 12 was the negative control).

Discussion

Non-typhoidal Salmonella from faeces of animals, working persons, contaminated milk containers, and farm environments can be the potential sources for milk contamination and subsequent transmission to humans [19]. Hence, it has to be noted that the detection of NTS in samples of these sources can be a risk for the spread of the pathogen to the public at large. In the current study, NTS was detected in 7.7% (95% CI=5.4-11.0%) of samples. Previously [20]. reported a prevalence of 6.6% of Salmonella among lactating cows and contact persons in dairy farms in Northwest Ethiopia. In Addis Ababa, a prevalence of 10.7% was reported [21]. among lactating cows and contact persons in dairy farms. On the other hand, a prevalence of 10.5% from dairy farms in and around Modjo town, Ethiopia, was also reported [22]. Another study reported a prevalence of 10.4% in samples from raw cow milk collected from dairy farms and households in Southern Ethiopia [23].

The emergence and spread of AMR in pathogens like NTS is an important challenge in the world, particularly in low-income countries, where the prevalence of bacterial pathogens in general and NTS, in particular, is high [24]. In this study, 89.3 % of the NTS isolates showed resistance to one or more classes of antimicrobials, and 46.4 % were MDR. The MDR strains are usually associated with higher morbidity (frequent bloodstream infections and hospitalizations) and mortality than susceptible strains, implying that the MDR isolates are more virulent than the susceptible ones [25-27]. In Ethiopia, the problem is frequently reported [28]. The meta-analysis study conducted by [29-30]. Reported that the prevalence of MDR among NTS isolates was 59.5 % and 65 %, respectively.

In this study, antimicrobial-resistant strains of NTS were detected in humans, animals, food (milk), and the environment which provided further evidence that AMR is not only an issue of human and animal health sectors but also the environment. Previous reports indicated that environmental waste (slurry) from dairy was one source of AMR and a critical point for the mitigation of AMR by prudent use of antimicrobials and appropriate waste management [31].

Multidrug-resistance strains are emerging as a result of continuous evolution and snowball effects, mainly due to misuse or overuse of antimicrobials. The overall proportion of MDR LFE in the current study was 70.7%. In line with this, the prevalence of MDR bacterial infection in Ethiopia was reported at 70.5% [32]. Another study reported a high prevalence of MDR bacterial infection (81.1%) in poultry farms in Gondar City, the Northwest part of Ethiopia [15]. The high prevalence of MDR is related to the widespread misuse of antimicrobials [33]. Inappropriate use of antimicrobials is due to limited awareness, low attitude and malpractices, which have been observed in the community, and among professionals in both human and animal health sectors [34-36]. The AMR drivers in the human dairy interface were also studied by [37] and found that animal antimicrobial use was positively linked with resistance in critical priority human pathogens and human antimicrobial use was positively linked with animal AMR in the interface.

The treatment of infection due to E. coli is challenging if the organism contains MDR character. In this study, 72.5% of the E. coli isolates were MDR. Previously, the proportion of MDR E. coli isolates was reported as 70.0% in Ethiopia [30]. Another study reported a 78.2% MDR prevalence of E. coli isolates from diarrheic patients in Ethiopia [29]. On the other hand, [38] reported a 26.7% prevalence of MDR in E. coli isolates from cattle and their environment in smallholder livestock production systems in Ethiopia. The difference may be related to the variations in the intensity of antimicrobial use in different areas [39].

In this study, the proportion of MDR Citrobacter spp isolates was 65.5%. Previously, the proportion of MDR among Citrobacter spp was reported at 71% in Ethiopia [30]. A worldwide rise of MDR problems among Citrobacter spp was also reported [40]. Among

Klebsiella spp isolates, the proportion of MDR was 60.0% which was slightly lower than the previous report (68%) in Ethiopia [30]. Seventy-three percent of Enterobacter spp isolates showed MDR characteristics. Multi-drug resistant Enterobacter spp infections are emerging in many parts of the world and a high prevalence (63.1%) of MDR among Enterobacter spp was also reported in Iran [41].

In the current study, the overall proportion of ESBL-producing LFE was 21.3%. Similarly, the proportion of ESBL enzyme-producing Enterobacteriaceae isolated from food handlers at the University of Gondar, northwest Ethiopia, was 21.7% [42]. Another report showed that the prevalence of ESBL-producing Enterobacteriaceae in Ethiopia was 30% [16-43]. The prevalence of ESBL in Africa was 29.3% among Enterobacteriaceae isolated from wastewater, being highest among isolates from hospital waste. They also reported that the prevalence of ESBL-producing Enterobacteriaceae in samples from farm and slaughterhouse sewage was 18.4%. These are all indicators that ESBL enzyme production among bacterial isolates is increasing and becoming a challenge for the treatment of bacterial infections. Data of the current study also showed that all ESBL producer bacterial isolates were MDR which indicated that ESBL production is creating more chances for the organism to resist antimicrobials. A high proportion of MDR (85.7%) among ESBL producers was also reported in India [44]. In addition, it had been observed that ESBL producers were proportionally more resistant to several antimicrobials than non-producers. Doxycycline was an exception which didn't show such characteristics. Previously, Sandhu [45-46]. reported that doxycycline exhibited efficacy for MDR isolates.

The ESBL production was detected in all common LFEs in this study. The proportion of ESBL enzyme-producing bacterial isolates were 29.7, 21.3, 16.4 and 16.0% among Enterobacter spp, E. coli, Citrobacter spp and Klebsiella spp, respectively. Previously, a higher pooled proportion of ESBL enzyme production among Klebsiella spp (61.8%), E. coli (41.2%) and other Gram-negative (41.9%) bacterial isolates either from human or animal were reported in Ethiopia and a 42.8% prevalence of ESBL-producing E. coli in dairy farms was reported in Egypt [47-48].

In this study, the proportion of ESBL-producing bacteria was considerably higher among bacteria isolated from raw milk. This is a risk for consumers and emphasizes the need to increase hygienic milk production and proper treatment before consumption [42]. found that the isolation of ESBL enzyme-producing Enterobacteriaceae among food handlers was associated with the consumption of unpasteurized milk. A high proportion of ESBL enzyme-producing bacteria in milk samples as compared to faecal and environmental samples was also reported in Malaysia [49].

It is a common practice to use farm sewage for fertilizer or other activities without appropriate waste management which can be the source of pathogenic bacteria and AMR strains. In this study, 91.2% of sewage samples were positive for LFE and of these, 89.5% were E. coli. Additionally, the farm sewage samples harbour MDR (75.5%) and ESBL-producing Enterobacteriaceae (18.9%). The prevalence of ESBL enzyme production among Enterobacteriaceae in wastewater was 24.8% [43]. Another report in Nigeria indicated that the waste from the farm was one source of ESBL enzyme-producing bacterial contamination of vegetables [50]. Therefore, this evidence indicated that waste from farms can be the source of not only infectious agents but also AMR bacteria which strengthens the need for appropriate waste management and disposal in dairy farms.

The genes encoding for ESBL enzymes are usually localized in plasmids, which makes them easily transferable among bacteria. The first □-lactamase was found in E. coli and termed TEM-1 after the name of the patient in which it was originally discovered (Temoneira) in Europe [51]. Before 1998, the dominant genes were blaTEM and blaSHV types. However, through mutation and gene exchanges, the diversity and the spectrum of β-lactamases expanded and several subfamilies and other families like blaCTX-M have been discovered in recent years and become dominant in many parts of the world [52]. In this study, blaCTX-M was found dominantly which was followed by blaTEM type. In line with this[49]. reported that blaCTX-M and blaTEM genes were predominantly detected in samples from dairy cows, milk and farm environments in Malaysia. Other reports also showed the dominance of blaCTX-M [43-54].

Nowadays, it is becoming common to divide the blaCTX-M family into several subgroups. In this study, the subtype blaCTX-M-15 was the most common (61.5%). The dominancy of blaCTX-M-15 was also reported by [55] and the clonal spread of blaCTX-M-15 as an outbreak in a rural Ethiopian hospital was also reported by [56]. The proportion of the blaSHV gene was the lowest (5.0%) in this study. An equivalent proportion (8.1%) of the blaSHV gene was also reported in E. coli isolated from beef cattle in China [57]. Other studies also reported low or no detection of the blaSHV gene [44-49].

Conclusions

Non-typhoidal Salmonella was detected in all types of samples (cow's faeces, milk and its container, human stool, hand swabs and dairy farm sewage). The proportion of NTS was high at the farm level which demonstrates that NTS is circulating in the human-dairy interface. The NTS isolates were more resistant to ampicillin, chloramphenicol, and tetracycline. However, the NTS isolates were more susceptible to ceftriaxone, norfloxacin, and azithromycin. The proportion of LFE was high, and isolates have high MDR, and ESBL production ability, which signifies the human-dairy interface is one of the important reservoirs and sources of AMR traits. The blaCTX-M and blaTEM were the most frequently detected genes in the ESBL-producing isolates. The output of this study is very important to design strategies for screening MDR and ESBL in different samples and provide more clues for developing standardized approaches for managing patients affected by ESBL-producing Enterobacteriaceae.

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