Antimicrobial resistance status of selected bacteria isolated from animal source foods and feed in Ethiopia

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Abstract

Antimicrobial resistance (AMR) of major food-borne pathogens has become an increasing public health problem worldwide. A cross-sectional study was conducted from August 2019 to July 2021 in high-potential meat and dairy products and commercial animal feed supply chain areas of Ethiopia. The objectives of the study was assessing AMR profile of target bacterial pathogens isolated from animal sources foods (ASFs) and feed. A total of 642 ASFs and feed samples collected from selected sampling sites were examined at the microbiology laboratory of animal products, veterinary drugs, and feed quality assessment center.Bacterial identification and antimicrobial susceptibility test (AST) were conducted using an automated Vitek 2 XL compact system. Out of 642 investigated samples, 24 different genera and 59 species of bacteria were identified. A total of 185 samples were positive for target bacteria of *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella* Species. The AST results showed AMR of target bacteria isolates against some of the tested antimicrobials. Of these, 83%, 55%, and 92% isolates of *Staphylococcus aureus, Escherichia coli,* and *Salmonella* Species, showed high level of AMR to Benzylpenicillin, Tetracycline, and Cefalexin/Gentamicin, respectively. The target bacteria isolated from ASFs and feed demonstrated multidrug resistance against some of the tested antimicrobials having public and veterinary importance. This reflects that ASFs and feed could serve as one of the sources for the spread and transmission of antimicrobial-resistant bacterial pathogens. Hence, there is a need for improv-

ing hygiene and sanitation practices along the ASFs and feed supply chains. Besides raising community awareness about the risks of AMR, emphasis on the rational use of antimicrobials in animal health practice and further investigations on AMR are recommended.
Keywords: Animal source foods; Antimicrobial resistance; Ethiopia; Feed; Target bacteria.

Introduction

The antimicrobial resistance (AMR) of major food-borne pathogens has become an increasing public health problem worldwide (Ferri *et al*., 2017). Though AMR is attributed to multiple factors, the contribution of the expanding use of antimicrobials in food animals has been considered the main reason for the worldwide rapid increase of AMR (WHO, 2015).

The growing worldwide phenomenon of AMR is generally associated with the improper use, overuse, or misuse of antimicrobials in humans and animals (Ferri *et al*., 2017) and agriculture (Martinez, 2009) which could enhance selective pressure for resistant strains. The resistant strains in the gut of animals and humans could horizontally transfer genes to similar or different species (e.g. *Salmonella* to *Escherichia coli*) (Martinez, 2009; Von Wintersdorff *et al*., 2016). Thus, humans can get infected by these resistant strains through the consumption of contaminated food of animal origin or through direct or indirect contact (Ghanbarpour *et al*., 2020).

Some reports indicate that major pathogens isolated from animal source food (ASF) and feed are resistant to antimicrobial agents such as quinolones, penicillin, aminoglycosides, macrolides, and tetracyclines (Mache, 2002; Ampaire *et al*., 2016; Mulu *et al*., 2017; Solomon *et al*., 2017; Waseem *et al*., 2019; Dahlin, 2020), but these antibiotics are still being used widely in the livestock sector for various purposes such as growth promotion (Tang *et al*., 2019). This increase in the use of antibiotics is mainly to satisfy the need for ASF attributed to the human population, income growth, and urbanization (Abegaz *et al*., 2018; Van *et al*., 2020).

In Ethiopia, farm hygiene, biosecurity, diagnostic capability, monitoring, tracing, and notification of pathogens, and AMR at the farm and national level are not well developed and uncontrolled antibiotic use and misuse are very com-

mon (Abdi *et al*., 2017). Thus, major pathogens such as *Escherichia coli*, *Salmonella* Species (Spps), *Staphylococcus aureus, Listeria monocytogene*s, and *Campylobacter* Spps could pose a public health problem (Molins *et al*., 2001). Therefore, AMR surveillance of pathogens in the ASFs and feed supply chain is crucial in combating AMR, guiding risk management and policy actions. Hence, in the present study, surveillance of the AMR profile of selected target bacterial pathogens isolated from ASFs and feed was conducted.

Materials and methods

Study design and study areas

A cross-sectional study was conducted from August 2019 to July 2021 in the potential meat and dairy products, and commercial animal feed supply chain areas of Ethiopia. Milk samples were collected from individual dairy cattle farms and milk collectors residing in Bishoftu, Sebeta, and Sululta towns, and meat samples were collected from separate butchers and/or municipal abattoirs in Addis Ababa, Bishoftu, Dukem, Modjo, and Sebeta towns while feed samples were collected from commercial feed manufacturing plants and distributors located in Addis Ababa, Adama, Bahirdar, Bishoftu, Burayu, Debratebor, Dessie, Injibara, Gelan and Sekota towns. The study areas were selected purposively based on the accessibility of sampling sites of commercial ASFs and feed having relatively high local market demand.

Sample size and sampling techniques

A total of 642 ASFs and feed samples were collected from sampling sites following Ethiopian Standard Agency (ESA) (ESA, 2016) and European Food Safety Authority (EFSA) standards (EFSA, 2010). The sample size was determined based on the testing capacity of the laboratory. In brief, 203 cow raw milk samples were collected from distinct dairy farms and milk selling points. Whereas, 256 meat/ carcass swab samples of cattle, sheep, goat, and chicken were obtained from selected butcher houses and municipal abattoirs. In addition, 174 feed samples were gathered from feed manufacturing plants and distributors in different parts of the country. Each sample was collected in a properly labeled sterile container following the standard sampling protocols and packed carefully to avoid leakage and cross-contamination (Roberts and Greenwood, 2008). The weight of samples collected was about 500g, 500 ml, and 1 Kg for meat, milk, and feed samples, respectively. The meat and milk are transported

under a cold chain and analyzed immediately. The samples were transported to the Ethiopia agriculture authority, microbiology laboratory of animal products, veterinary drug and feed quality assessment center and stored under a cold chain (EFSA, 2010; ESA, 2016).

Target bacterial culturing, isolation, and identification techniques

The techniques recommended by the International organization for standardization (ISO) (ISO, 2002) and ESA (ESA, 2012) were employed for the sample preparation and morphological characterization of target bacteria colonies. Further confirmation of the target bacterial isolation was performed by a fully automated Vitek 2XL compact system following the manufacturer method (BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b).

Staphylococcus aureus (S. aureus) and *Escherichia coli* (*E. coli*) identification

About 10 g of feed and meat samples, 10 x 10 cm of meat carcass swab, and 10 ml of milk samples were pre-enriched in 90 ml of sterile buffered peptone water (BPW) to yield a 1/10 dilution, then homogenized using a smasher, and incubated at 37°C for 24 hrs (Sandel and McKillip, 2004; ESA, 2012). For *E. coli* isolation, a loop full of the incubated culture was streaked onto MacConkey agar and then incubated at 37°C for 24 hrs. A single pink colony was taken from cultured MacConkey agar and sub-cultured on Eosin Methylene Blue (EMB) agar and then incubated at 37°C for 24hrs. Colonies showing metallic sheen on EMB were sub-cultured on nutrient agar, incubated at 37°C for 24 hrs, and finally confirmed by Vitek 2XL compact system using Gram Negative (GN) cards having 47 test substrates. Similarly, for *S. aureus* isolation, a loop full of suspension was taken from incubated BPW culture and cultured on blood agar, and then incubated at 37°C for 24 hrs. A single pink colony was taken from incubated blood agar and sub-cultured on Mannitol Salt Agar (MSA) and then incubated at 37°C for 24 hrs. Colonies showing golden yellowish on MSA were sub-cultured on nutrient agar, incubated at 37°C for 24 hrs and confirmed by automated Vitek 2XL compact system using Gram-Positive (GP) cards (Sandel and McKillip, 2004; BioMérieux, 2011; ES ISO, 2012; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c).

Salmonella Species *identification*

About 25 g of feed and meat samples, 25 ml of milk samples, and 10 x 10 cm meat carcasses swabs were pre-enriched in 225 ml of sterile BPW for feed and milk samples, and 90 ml of sterile BPW for swabs samples to yield a 1/10 dilution, then homogenized using smasher and incubated at 37°C for 24 hrs. A 0.1 ml and 1ml of the incubated BPW culture were transferred into 10ml of Rappaport Vassiliadis Medium with Soya (RVS) and Muller-Kauffmann Tetrathionate (MKTT) broth respectively as selective enrichment and inoculated at 41.5°C for RVS and 37 °C for MKTT for 24 hrs. A loop full of the inoculated RVS and MKTT broth was cultured onto Xylose Lycine Deoxycholate (XLD) agar which is selective media and incubated at 37°C for 24-48 hrs (ES ISO, 2002). Reddish/pink colonies of *Salmonella* on XLD plates were sub-cultured on Nutrient Agar, incubated at 37°C for 24 hrs and confirmed by using Vitek 2XL compact system according to the manufacturer method (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c).

Confirmatory identification of target bacteria species by Vitek 2XL compact system

The Vitek 2 GN and Vitek 2 GP identification cards were used for confirmatory identification of GN and GP bacteria, respectively by Vitek 2XL compact system according to the manufacturer's recommendations (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a). In brief, 2–3 pure fresh colonies taken from incubated nutrient agar were suspended in 3.0 ml of sterilized saline and thoroughly mixed to have 0.5 to 0.63McFarland turbidity (BioMérieux, 2020c). The bacteria in the suspension were identified using Vitek 2XL compact system (BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b; BioMérieux, 2020c). The results were interpreted by the Vitek database at different confidence levels or probabilities as excellent (96-99%), very good (93-95%), good (89-92%), acceptable (85-88%), none or low reactive/discrimination biopattern and unidentified microorganisms. Bacteria identification results were considered acceptable when the confidence level is ≥85% probability. The final identification results were obtained automatically approximately 8 hrs or less for GP bacteria and 10 hrs or less for GN bacteria (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a; BioMérieux, 2020b).

Antimicrobial susceptibility test (AST)

AST against selected antimicrobial agents were conducted for each of isolated target bacteria (*S.aureus, E.coli* and *Salmonella* Spps) according to the Vitek 2XL compact system protocol (BioMérieux, 2011; BioMérieux, 2022a; BioMérieux, 2020b). Eight antimicrobial classes containing a total of 23 antimicrobial agents coated with Vitek 2 AST-GN96 card and Vitek 2 AST-GP79 cards were used (Table 1). The selection criteria of the antimicrobials were based on the Office International des Epizooties (OIE) list of antimicrobial agents of veterinary importance (OIE, 2015).

The pure colonies of the identified *S. aureus, E.coli* and *Salmonella* Spps were suspended in 3.0 ml of sterilized saline using different sterile test tubes and thoroughly mixed (BioMérieux, 2011; BioMérieux, 2022a; BioMérieux, 2020b). The turbidity of the bacterial suspensions was adjusted with a DensiChekPlus meter to match that of a McFarland 0.5–0.63 standard (BioMérieux, 2020c). Then after, AST cards were selected based on the gram characteristics of the bacteria isolates (AST GP card for *S.aurues* and AST GN card for *E.coli* and *Salmonella*), then loaded into the Vitek XL compact system for automatic processing. The results were interpreted by the Vitek and obtained automatically. Susceptibility test results of the bacteria were supposed to be observed in less than 19 hrs (Pincus, 2006; BioMérieux, 2011; BioMérieux, 2020a).

The break-point of antimicrobial is expressed numerically in µg/ml as minimum inhibitory concentration (MIC) for AST/AMR interpretation. AST results in interpretations were based on the guidelines of the Clinical and Laboratory Standards Institute (CLS) (CLSI, 2017). The results interpretations for AST/AMR obtained from Vitek 2XL compact system were categorized as Susceptible (S), Intermediate (I) and Resistant (R) (BioMérieux, 2020a; BioMérieux, 2020b).

Table1. Target bacteria pathogens-antimicrobial agent combinations used for antimicrobial susceptibility test study.

Target bacteria	Antimicrobial class	Antimicrobial agents (AMA)	AST card coated with AMA	AMA importance in veterinary***
Salmonella and E.coli	1. Quinolones	Flumequine	AST-GN	VHIA
		Marbofloxacin	AST-GN	VCIA
		Enrofloxacin**	Both	
	2. Beta-lactams			
	2.1 Cephalosporins	Cefalexin	AST-GN	VHIA
		Cefalotin**	Both	
		Cefoperazone	AST-GN	VCIA
		Ceftiofur**	Both	
		Cefquinome**	Both	
	2.2 Penicillins	Amoxicillin/ Clavulanic acid/	AST-GN	VCIA
S.aureus		Ampicillin**	Both	
		Benzylpenicillin*	AST-GP	
		Oxacillin*	AST-GP	
	3. Aminoglycosides	Gentamycin**	Both	VCIA
		Neomycin**	Both	
		Amikacin	AST-GP	
		Kanamycin	AST-GP	
	4. Macrolides	Erythromycin	AST-GP	VCIA
		Tilmicosin	AST-GP	
		Tylosin	AST-GP	
	5. Lincosamides	Clindamycin	AST-GP	VHIA
	6. Tetracyclines	Tetracycline**	Both	VCIA
	7. Sulfonamide and Diaminopyrimidines/ Trimethoprim	Trimethoprim/ Sulfamethoxazole**	Both	VCIA
	8. Amphenicols	Florfenicol**	Both	VCIA

*Benzylpenicillin and *Oxacillin antimicrobial agents were used only for *S.aureus.*

**Antimicrobial agents coated with both AST-GP and AST-GN cards were used for *S.aureus, E.coli,* and *Salmonella* Spps.

***Importance of antimicrobial agents in veterinary (i) Veterinary critically important antimicrobial agents (VCIH) (ii) Veterinary highly important antimicrobial agents (VHIA) (iii)Veterinary important antimicrobial agents(VIA) (OIE, 2015).

Data management and analysis

Microsoft® Excel (2010) was used for data management and analysis. Data generated from laboratory investigations were coded, entered, and/ or calculated on an MS Excel spreadsheet. The percentage of occurrence of bacteria and /or target bacteria Spps isolated from the ASF and feed was calculated as the number of positive (confirmed) samples divided by the total number of samples investigated (processed) in the laboratory. Similarly, the percentage of AST profiles (AMR development) of target bacteria was calculated as the number of target bacteria isolates that showed resistance against tested AMA divided by the total number of target bacteria tested for AST against selected AMA.

Results

Out of 642 investigated ASFs and feed samples, 24 different genera and 59 species of bacteria were identified from morphologically known target bacteria colonies loaded into the Vitek 2XL compact system using GP and GN identification cards. All the identified GP and GN bacteria are depicted in Table 2.

Table 2. Bacteria genera and species identified from the test samples by gram positive and gram negative cards.

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
I	Gram-negative bacteria				
1.0	***Escherichia coli***	24 (20.70)	77 (29.00)	0 (0.00)	101 (15.70)
2.0	***Salmonella*** **Group**				
2.1	*S.Typhi*	1 (0.49)	1(0.37)	0 (0.00)	2 (0.31)
2.2	*S.enterica diarizonae*	1 (0.49)	7(2.64)	2 (1.15)	10 (1.56)
3.0	***Klebsiella*** **Species**				
3.1	*K. oxytoca*	7(3.45)	5 (1.88)	0 (0.00)	12 (1.87)
3.2	*K. pneumoniae*	3 (1.47)	4 (1.50)	3 (1.72)	10 (1.56)
4.0	***Shigella*** **Group**	2 (0.98)	1 (0.37)	0 (0.00)	3 (0.47)
5.0	***Proteus*** **Species**				
5.1	*P.mirabilis*	4 (1.97)	12 (4.53)	2 (1.15)	18 (2.80)
5.2	*P.vulgaris*	0 (0.00)	3 (1.13)	0 (0.00)	3 (0.47)
5.3	*P.hauseri*	0 (0.00)	1 (0.37)	0 (0.00)	1(0.15)

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
6.0	***Enterobacter* Species**				
6.1	*E.aerogenes*	15 (7.39)	15 (5.66)	8 (4.60)	38 (5.92)
6.2	*E.cloacae complex*	14 (6.90)	8 (3.02)	8 (4.60)	30 (4.67)
6.3	*E.cloacae dissolvents*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
6.4	*E./Kluyveria /intermedia*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
7.0	***Morganella* Species**				
7.1	*M. morgenisspmorgeni*	1 (0.49)	2 (0.75)	0 (0.00)	3 (0.47)
8.0	***Raultella* Species**				
8.1	*R.planticola*	4 (1.97)	0 (0.00)	0 (0.00)	4 (0.62)
8.2	*R.ornithinoltyica*	10 (4.92)	18 (6.79)	7 (4.02)	35 (5.45)
9.0	***Pantoe* Species**	4 (1.97)	1 (0.37)	1 (0.57)	6 (0.93)
10.0	***Pseudomonas* Species**				
10.1	*P.aeroginosa*	3 (1.47)	12 (4.52)	0 (0.00)	15 (2.33)
10.2	*P.putida*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
11.0	***Serratia* Group**				
11.1	*S.fonticola*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
11.2	*S.mercescense*	0 (0.00)	1 (0.37)	0 (0.00))	1 (0.15)
11.3	*S.liquefaciences*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
11.4	*S.plymuthica*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
11.5	*S.odorifera*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
12.0	***Citrobacter* Species**				
12.1	*C.freundii*	2 (0.98)	16 (6.03)	1 (0.57)	19 (2.96)
12.2	*C.sedlaki*	0 (0.00)	0 (0.00)	1 (0.57)	1 (0.15)
12.3	*C.braakii*	0 (0.00)	3 (1.13)	0 (0.00)	3 (0.47)
12.4	*C.werkmanii*	1 (0.49)	1 (0.37)	0 (0.00)	2 (0.31)
13.0	***Providencia* Species**				
13.1	*P.stuartii*	0 (0.00)	1(0.37)	0 (0.00)	1 (0.15)
13.2	*P.rettgeri*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
14.0	***Cedecea* Species**				
14.1	*C.davisae*	0 (0.00)	1(0.37)	0 (0.00)	1 (0.15)
15.0	***Burkholderia* Species**				
15.1	*B.cepacia group*	2 (0.98)	1 (0.37)	1 (0.57)	3 (0.47)
16.0	***Sphingomonadacea* Species**				

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
16.1	*S.paucimobilis*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
17.0	***Aeromonas* Species**				
17.1	A.hyrdophila/punctata	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
17.2	*A.salmonicida*	2 (0.98)	0 (0.00)	0 (0.00)	2 (0.31)
18.0	***Pasteurella* Species**				
18.1	*P. canis*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
II	**Gram-positive bacteria**				
1.0	***Staphylococcus* Species**				
1.1	*S.aureus*	46 (22.66)	26 (9.81)	0 (0.00)	72 (11.21)
1.2	*S.saprophyticus*	1 (0.49)	2 (0.75)	0 (0.00)	3 (0.47)
1.3	*S.xylosus*	2 (0.98)	1 (0.37)	0 (0.00)	3 (0.47)
1.4	*S.sciuri*	5 (2.46)	23 (8.68)	0 (0.00)	28 (4.36)
1.5	*S.gallinarum*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
1.6	*S.lentus*	7 (3.45)	7 (2.64)	0 (0.00)	14 (2.18)
1.7	*S.intermedius*	1 (0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.8	*S.vitulinus*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
1.9	*S.hominis*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
1.10	*S.warneri*	0 (0.00)	7 (2.64)	0 (0.00)	7 (1.09)
1.11	*S.chromogenes*	2 (0.98)	0 (0.00)	0 (0.00)	2 (0.31)
1.12	*S.haemolyticus*	1(0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.13	*S.cohnii spp cohnii*	1(0.49)	0 (0.00)	0 (0.00)	1 (0.15)
1.14	*S.simulans*	0 (0.00)	3(1.13)	0 (0.00)	3 (0.47)
2.0	***Enterococcus* Species**				
2.1	*E.faecium*	2 (0.98)	0 (0.00)	2 (1.15)	4 (0.62)
2.2	E.faecalis	1 (0.49)	12 (4.52)	0 (0.00)	13 (2.02)
2.3	*E.gallinarium*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
3.0	***Aerococcus* Species**				
3.1	*A.viridans*	2 (0.98)	2 (0.75)	1 (0.57)	5 (0.77)
4.0	*Leuconostoc species*				
4.1	*L.mesentero*	9 (4.43)	3 (1.13)	2 (1.15)	14 (2.18)
5.0	***Streptococcus* Species**				
5.1	*S.pseudoporcinus*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)
5.2	*S.thoraltensis*	0 (0.00)	1 (0.37)	0 (0.00)	1 (0.15)

S/N	Identified bacteria genera and species types	No (%) of occurrence bacteria species			
		Milk (n=203)	Meat (n=265)	Feed (n=174)	Total (N=642)
6.0	*Kocuria species*				
6.1	*K.kristinae*	0 (0.00)	4 (1.5)	0 (0.00)	4 (0.62)
6.2	*K.rhizophila*	0 (0.00)	2 (0.75)	0 (0.00)	2 (0.31)
III	Mixed bacteria isolation	45 (22.17)	57 (21.50)	5 (3.40)	107 (16.6)

Of all the tested samples, 185 were positive for the three target bacterial pathogens; *S. aureus* 11.2% (72 out of 642), *E. coli* 15.7% (101 out of 642), and *Salmonella* Spps 1.9% (12 out of 642) (Table 3).

Table 3. Target bacteria species occurrence on a sample basis.

Sample type	Tested sample number	No (%) of the target bacteria isolates/species			
		S. aureus	***E. coli***	***Salmonella* Spps**	**Total**
Raw milk	203	46 (20.0)	24 (11.8)	2 (1.0)	72 (35.5)
Meat/carcass swabs	265	26 (9.8)	77 (29.1)	8 (3.0)	111 (41.9)
Feedstuffs	174	0 (0)	0 (0)	2 (1.1)	2 (1.1)
Total	642	72 (11.2)	101(15.7)	12 (1.9)	185 (28.8)

Antimicrobial susceptibility test (AST)

The AST was conducted on all the identified target bacterial species. The AST result of *S. aureus, E. coli,* and *Salmonella* bacteria isolates tested against sixteen (16), fourteen (14), and fifteen (15) AMA, respectively shows high resistance to some antibiotics and no resistance (susceptible) to some antibiotics. The AMR development levels of *S. aureus*, *E. coli,* and *Salmonella* Spps against the selected antimicrobials are summarized and presented in Figures 1, 2, and 3, respectively.

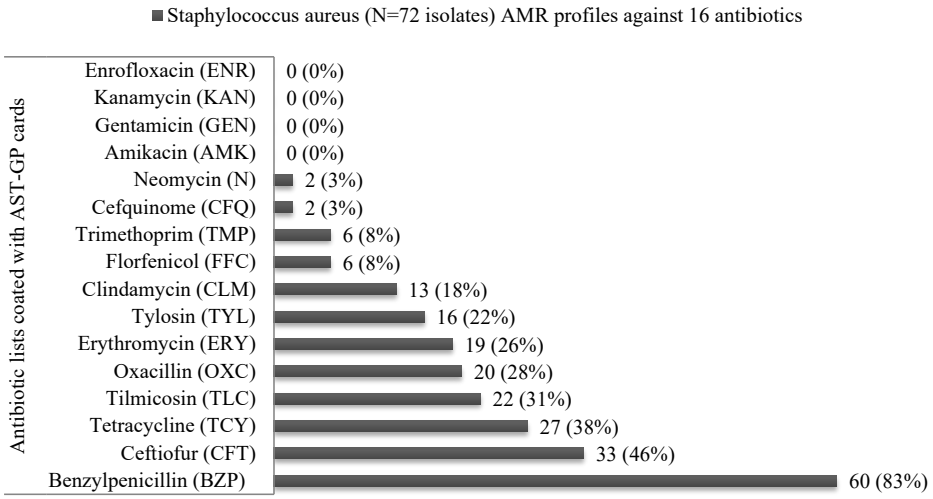

Figure 1. *Staphylococcus aureus* AMR development levels against selected antibiotics.

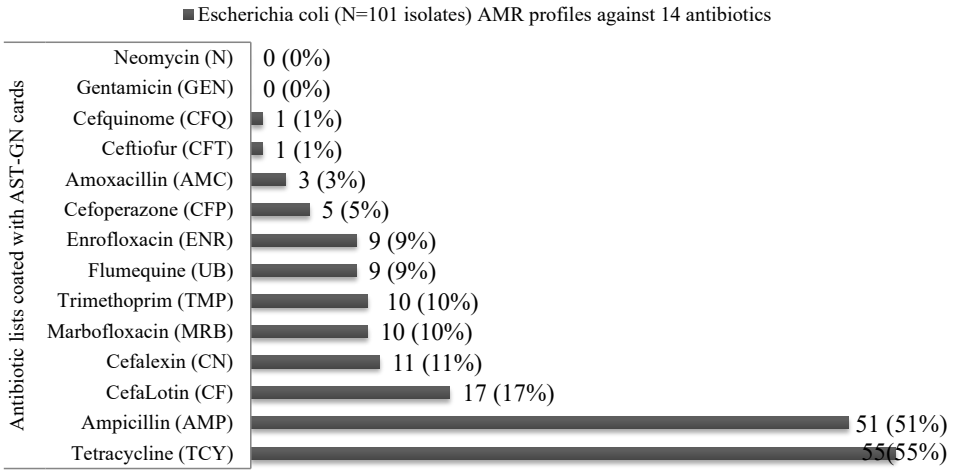

Figure 2. *Escherichia coli* AMR development levels against selected antibiotics.

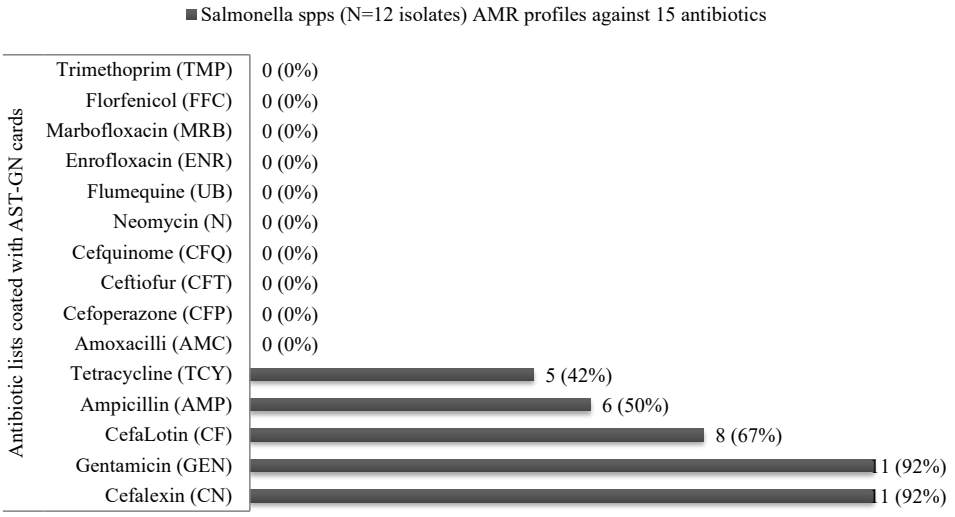

Figure 3. *Salmonella* Spps AMR development levels against selected antibiotics.

Discussion

In the present study, a total of 642 samples consisting of 203 raw milk, 265 meat/carcass swabs, and 174 feedstuffs were investigated for the presence of target bacteria and AST profiles. The findings proved the presence of 24 different genera and 59 species of bacteria in the investigated samples with the overall high prevalence of *E.coli* 15.7% (101 out of 642) followed by *S. aureus* 11.2% (72 out of 642) and *Salmonella* Spps 1.9% (12 out of 642). This implies that ASFs and feed samples obtained from the study areas could contribute as the reservoirs for strains of *S. aureus*, *E. coli,* and *Salmonella* Spps and point to the presence of poor food hygiene and sanitation management practices. All isolates of target bacterial Spps were tested for AST against the AMA having different levels of veterinary and public health importance.

The AST results observed in the present study suggest resistance of *S. aureus* for the majority (75%) of the tested antimicrobials except for Amikacin, Gentamycin, Kanamycin, and Enrofloxacin. The *S. aureus* bacteria recovered from milk and meat samples demonstrated a relatively high level of resistance to

Benzylpenicillin (83%), Tetracycline (38%), and Erythromycin (26%). Similar patterns of resistance were reported in a previous study conducted in Bishoftu, Ethiopia which indicates the resistance of 18 *S.aureus* isolated from 253 samples of meat and dairy milk to Penicillin (94.45%), Tetracycline (27.8 %), and Erythromycin (33.33%) (Matewos, 2020). Also, the resistance of *S. aureus* isolates to Oxacillin (28%) and Clindamycin (18%), observed in the current study was in accordance with the findings reported for Oxacillin (31%) and Clindamycin (13.8%) (Reta *et al*., 2016). The findings observed for *S. aureus* indicated that ASFs and feed being supplied to the market in the study areas could potentially be a threat to both public and animal health as well as the environment. The resistance of *S. aureus* to clindamycin; a drug that is not used in veterinary practice in Ethiopia suggests the transfer of genes of resistant strain among the environment, livestock, and human. All *S. aureus* isolates are found to be 100% susceptible to Amikacin, Gentamycin, and Kanamycin antimicrobials agents and suggesting that they are relatively the most effective drugs *in-vitro*.

E. coli isolates obtained from milk and meat samples are non-responsive to Tetracycline (55%), Ampicillin (51%), Cefalotin (17%), Cefalexin (11%), Marbofloxacin (10%), Trimethoprim (10%), Flumequine (9%) and Enrofloxacin (9%). In previous studies, a relatively low proportion of *E. coli* isolates show resistance against Tetracycline (8.1%), Ampicillin (21.5%), and Trimethoprim (4.6%) (Mwanyika *et al*., 2016) and relatively high resistance of *E. coli* O157:H7against Tetracycline (81.8%) is reported (Mohamed *et al*., 2020). This might be due to differences among different environments and hygiene practices.

In contrast to our finding, high resistance rates of *E. coli* were observed (>60%) for Flumequine, while resistance reported for Enrofloxacin and Marbofloxacin are almost similar (<40%) (Vanni *et al*., 2014).

In the current study, the AST results of *Salmonella* Spps isolated from milk, meat and feed samples conducted using fifteen (15) selected antimicrobials coated with GN cards indicated more resistance to Cefalexin (92%), Gentamicin (92%), Cefalotin (67%), Ampicillin (50%) and Tetracycline (42%) as compared to the findings reported in the previous study (Ejo *et al*., 2016; Okorie-Kanu *et al*., 2016) in which more than 90-100% of *Salmonella* Spps demonstrate resistance to Tetracycline. A study conducted in Addis Ababa (Alemu *et al*., 2011) showed that *Salmonella* Spps isolated from dairy lactating cows demonstrate

resistance to Ampicillin (100%) and Tetracycline (33.33%). The findings of the present study reveal that *Salmonella* Spps showed no resistance to Amoxicillin, Cefoperazone, Ceftiofur, cefquinome, Neomycin, Flumequine, Enrofloxacillin, Marbofloxacin, Florfenicol, and Trimethoprim. This might be due to the low frequency of use in the study area in veterinary services, and perhaps in human medicine.

The limitation of this study is that the knowledge, attitude, and practice (KAP) of personnel working in the municipal abattoir, dairy farm, and feed manufacturing plant were not captured during sample collection. Besides, due to the shortage of AMA-coated test cards, the AST tests were conducted for only prioritized target pathogenic bacteria.

Conclusions

Overall, the findings of the present study reveal a high level of AMR for some antimicrobials having different levels of public and veterinary importance against target bacteria isolated from ASFs and feed. This implies that ASFs and feedstuffs in the study areas could potentially be a reservoir of drug-resistant bacteria dissemination and transmission. Thus, consumption of ASFs and feedstuffs could present public and veterinary health risks. In addition, since a large proportion of the population in Ethiopia lives near animals, the findings suggest a high possibility of transmission of resistant microorganisms from animals to humans and vice versa. This reflects that there needs to be a strict medicine use regulatory activity and improved food hygiene and sanitation management practices along the ASFs and feed supply chains.

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