

# Distribution of drug resistance among enterococci and *Salmonella* from poultry and cattle in Ethiopia

Behailu Bekele · Mogessie Ashenafi

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**Abstract** Enterococci and *Salmonella* were isolated from feces of chicken in intensive poultry farms and cattle which are maintained following traditional practices. Their resistance to different antibiotics was also determined. A total of 298 enterococcal isolates consisting of *Enterococcus faecium* (49.6%), *Enterococcus durans* (26.9%), *Enterococcus hirea* (11.9%), and *Enterococcus faecalis* (11.5%) were obtained. Among the enterococci, resistance to erythromycin (Ery), clindamycin (Cli), amoxicillin (Amo), ampicillin (Amp), and cephalothin (Cep) was high. Resistance to vancomycin (Van) was detected in all enterococcal species. Over 80% of the isolates showed multiple drug resistance. The most dominant patterns in poultry were Amo/Amp/Cep/Pen and Amo/Amp/Cep/Cli/Pen/Van. Among isolates from cattle, Amo/Amp/Cep/Cli/Ery/Pen/Van and Amo/

Amp/Cli/Ery/Pen/Van constituted the most dominant multiple resistance patterns. A total of 51 *Salmonella* isolates were obtained from poultry (43/280) and cattle (8/450). About 70% of the isolates had varying resistance to the tested antibiotics. Multiple drug resistance was observed in over 30% of the *Salmonella* isolates. The most frequent resistance pattern was Amo/Amp/Cip/Gen/Str in cattle and Amo/Amp/Cep/Cip/Gen/Kan/Str in poultry. Enterococcal and *Salmonella* isolates showed multiple resistance to those antibiotics used in human and veterinary medicine. The high frequency of isolation of resistant enterococci is indicative of the wide dissemination of antibiotic resistant bacteria in the farm environment.

**Keywords** Enterococci · *Salmonella* · Multiple drug resistance · Poultry · Cattle

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B. Bekele  
Department of Biology, Addis Ababa University,  
P.O. Box 1176, Addis Ababa, Ethiopia

M. Ashenafi (✉)  
Institute of Pathobiology, Addis Ababa University,  
P.O. Box 1176, Addis Ababa, Ethiopia  
e-mail: mogessie@gmail.com

*Present Address:*

B. Bekele  
Department of Food technology, Bahir Dar University,  
P.O. Box 26, Bahir Dar, Ethiopia

## Introduction

Despite the shrinking in size of arable land due to the growing world population, the contribution of animal productions towards meeting the need for more food has been significant. However, microbiological safety of animal foods is becoming a major concern

More than 85% of the Ethiopian population is dependent on agriculture, and Ethiopia's livestock population is the largest in Africa with 30,000,000 cattle, 24,000,000 sheep, 18,000,000 goats, 7,000,000 equines, 1,000,000 camels, and 53,000,000 poultry

(Mengistu 2005). A larger segment of the rural and urban population is dependent on livestock for food and generation of income. Thus, many zoonotic bacterial pathogens can reach humans through consumption of contaminated foods and food products of animal origin and through close contact with the animals.

Over the years, bacterial pathogens have developed resistance to various antibiotics. The main risk factor for the increase in the antibiotic resistance is an extensive use of antibiotics in human health and agriculture (Lukasova and Sustackova 2003), which leads to the emergence and dissemination of resistant bacteria and resistant genes in animals and humans. The veterinary use of antibiotics includes therapy, prophylaxis, and growth promotion. Some antibiotic are used both in veterinary and human medicine. The antimicrobial agents used in animal care are important, not only in increasing the resistance in animal pathogens, but also in bacteria transmitted from animals to humans (Aarestrup et al. 2008).

The frequently exposed microbes during the use of antibiotics for these purposes are the gut flora and, hence, there is a possible development of resistance in the pathogenic and commensal bacteria. The commensal bacteria constitute a reservoir of resistant genes for possible transfer to pathogenic bacteria. Their level of resistance is considered as a good indicator of the selection pressure of antibiotic use and for resistance problem to be expected in pathogens (Bonomo and Rossolini 2008).

Enterococci are the normal flora of intestinal tract of humans and animals. The genus consists of more than 20 species, but fecal enterococci consist of mainly four species: *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans*, and *Enterococcus hirae* (Tejedor et al. 2001).

In human medicine, nosocomial and opportunistic infections caused by enterococci are becoming increasingly important problems, particularly those due to *E. faecalis* and *E. faecium* (Wassenaar and Silley 2008) with resistance to a number of antibiotics. Besides, enterococci serve as reservoir for transferable resistance genes to other pathogens (Sahlström et al. 2009).

There is considerable information on antimicrobial resistance in *Salmonella* of human and food animal origin in Ethiopia and it has emerged as a considerable public health concern worldwide (Molla et al.

2003). This is due to the possible transfer of resistant *Salmonella* and other zoonotic bacterial pathogens or their resistant genes to humans through consumption of contaminated food and food products (Van Hao et al. 2007).

The aim of this study was, therefore, to assess the distribution of antibiotic resistance among enterococcal and *Salmonella* isolates from feces of poultry in intensive poultry farms and cattle reared following traditional practices.

## Materials and methods

### Collections of samples

Two hundred eighty cloacal swabs and 450 fecal samples were collected from poultry and cattle, respectively. The poultry samples were obtained from cloaca of chicken using sterile swabs from four intensive farms. Samples from cattle (30 g) were collected from Addis Ababa and its surroundings in sterile Stomacher bags (Seward 400, UK). The samples were transported to the laboratory using icebox and were processed within 1–2 days after arrival to the laboratory.

### Microbiological analysis

Fecal samples collected from different poultry farms were streaked on Bile Esculin Agar (Oxoid). The plates were incubated at 37°C for 24 h. Colonies which were considered as presumptive *Enterococci* were further tested for the following characteristics.

Cell shape and arrangement was determined microscopically. Production of catalase was tested by flooding colonies with a 3% H<sub>2</sub>O<sub>2</sub> solution. Test for Gram reaction was carried out using potassium hydroxide test according to Gregerson (1978).

Growth at 6.5% NaCl was tested in Brain heart infusion broth (BHI) which contained 6.5% NaCl. The inoculated broth was then incubated for 24 h at 35°C. Growth was visually detected. Growth at 45°C was tested in BHI broth incubated at 45°C for 24 h. Growth was visually detected.

Tests for carbohydrate fermentation were performed in a broth basal medium containing phenol red as indicator. Mannitol, sorbitol, sorbose, raffinose, lactose, and arabinose were separately added to the

medium at 1% concentration. An overnight broth culture of each isolate was separately inoculated into each of the fermentation tubes and incubated at 37°C for 24 h. The result was considered positive when the broth turned yellow.

For *Salmonella*, rectal swabs from chicken were vortexed vigorously in buffered peptone water (1%) to suspend the contents. Fresh fecal samples from cattle were homogenized by vortexing in buffered peptone water (1%). These were incubated at 37°C for 18–24 h for primary enrichment. Selenite broth, tetrathionate broth, Rappaport-Vassiliadis, and maninitol selenite broth and Muller-Kauffman tetrathionate broth were prepared as secondary enrichment according the instruction of the manufacturer. Plating was done on MacConkey agar no 3, *Salmonella-Shigella* agar, and xylose lysine desoxycholate. All agar media and broths were from Oxoid.

Suspected *Salmonella* colonies were tested for the following biochemical tests. Triple sugar iron agar, lysine iron agar, urea agar, Simmons citrate agar, medium sulphur indole motility, mannitol broth, sucrose broth (1%), and glucose broth. Presumptive *Salmonella* isolates were further confirmed by using API 20E identification system as described by the manufacture (BioMerieux, France)

#### Drug susceptibility testing

Resistance was tested on Mueller–Hinton agar plates following the standardized disk diffusion technique (Jorgenson et al., 1999) with Oxoid drug disks: ampicillin (Amp; 10 µg), sulfamethoxazole/trimethoprim (SXT; 25 µg), penicillin G (Pen; 10iu), vancomycin (Van; 30 µg), erythromycin (Ery; 15 µg), doxycycline (Dox; 30 µg), streptomycin (Str; 10 µg), clindamycin (Cli; 2 µg), cephalothin (Cep; 30 µg), amoxicillin (Amx; 25 µg), and amikacin (Ami; 30 µg). All antibiotic disks were from Oxoid. For *Salmonella*, ciprofloxacin (Cip; 5 µg) was included but Cli, Ery, and Van were not used. The reference strains, *S. aureus* (ATCC 6538) and *E. coli* (ATCC 25922), sensitive to all the drugs used in this study, were routinely tested. Interpretation of readings as sensitive, intermediate, or resistant was made according to a chart (Jorgenson et al. 1999). Intermediate readings were few and, therefore, considered as sensitive for the purpose of assessing the data.

#### Data analysis

Descriptive statistics was used to compute percentage and mean. Analysis of variance was used to see variation in resistant isolate among farms and species for the different antibiotics tested. A difference was considered statistically significant when the *p* value was less than 0.05

#### Results

Of the 280 chickens sampled from four intensive poultry farms (farms A, B, C, and D), 234 yielded isolates consisting of *E. faecium* (49.6%), *E. durans* (26.9%), *E. hirea* (11.9%) and *E. faecalis* (11.5%; Table 1). Of the fecal samples collected from 450 cattle, 64 were positive for enterococci and consisted of *E. faecium* (34), *E. faecalis* (nine), and *E. durans* (21). *E. hirea* was not encountered in cattle.

The overall prevalence of *Salmonella* isolates from poultry was 15.4% (43/280). The prevalence in farms A, B, C, and D was 18.6% (13/70), 20% (14/70), 15.7% (11/70), and 7.1% (5/70), respectively. Out of 450 cattle fecal samples, only 1.8% (8/450) were positive for *Salmonella*

Of the 234 poultry isolates subjected to antimicrobial susceptibility testing, using a panel of 11 different antimicrobials, 40–80% of enterococci showed resistance to Pen, Cli, Cep, Amo, or Amp. Resistance to the other antimicrobials was less than 40%. All isolates of *E. faecalis* showed susceptibility to Ami. Van resistance was observed in the four species with a frequency between 30 and 54% (Table 2). Van resistance was observed in all poultry farms with varying frequencies. All isolates from farm D also showed resistance to Pen, Cli, and Cep (Table 2). There was a significant difference among farms in mean resistant isolates to Pen and Van ( $p < 0.05$ ).

Multiple drug resistance (MDR) to three or more antibiotics was seen in 78.2% of the enterococcal isolates. Resistance was noted to four, five, six, seven, and more antibiotics at varying proportions. A smaller percentage of isolates were also resistant to  $\geq 10$  or 11 antibiotics (Table 3).

*E. hirea* had the largest proportion of multiple drug resistance (91.2%). *E. faecalis*, *E. durans*, and *E. faecium* accounted for 81.5%, 79.4%, and 73.3%, respectively. Higher number of resistance pattern was

**Table 1** Percentage distribution of enterococcal isolates in the four farms

Farm	No. of samples	No. of isolates	Distribution in percentage			
			<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. durans</i>	<i>E. hirea</i>
A	70	66	51.5	12.1	22.7	13.6
B	70	60	50	11.6	25	13.3
C	70	67	49.2	11.9	29.8	8.9
D	70	41	46.3	9.7	31.7	12.2
Total	280	234	49.6	11.5	26.9	11.9

seen in *E. faecium* than in the other species (Table 3). MDR was observed in all four farms, but it was relatively higher in farms A and D (92% each). In farms B and C, the frequency of MDR was  $\leq 80\%$ .

Of the 64 enterococcal isolates from cattle, more than 70% showed resistance to Pen, Ery, Cli, or Amo. Resistance to Van was observed in all the three species isolated from cattle but was relatively higher among *E. faecalis* isolates. MDR was observed in 58 of the 64 enterococcal isolates. In *E. faecium* alone, multiple drug resistance was seen in 15 different

resistance patterns (data not shown) dominated by eight frequent patterns (Table 3)

Of the 65 *Salmonella* isolated from poultry tested for an array of eight different antimicrobials, 38 were resistant to Str, none was resistant to Ami, and the resistance to the other antimicrobials was less than 50%. Sixteen isolates from poultry showed MDR to three or more antimicrobials. None of the resistance patterns was dominant. Of the nine *Salmonella* isolates from cattle, three isolates were resistant to Str and Amo. Resistance to Kan, Cep, and Ami were

**Table 2** Distribution of drug resistance among enterococci in different farms

Farm	Species	No. of Isolates	Percent of resistance										
			Dox	Amo	Pen	Amp	Ami	Van	Ery	Str	Cli	Sxt	Cep
A	<i>E. faecium</i>	34	44	59	88	44	18	53	56	35	71	12	44
	<i>E. faecalis</i>	8	13	63	88	25	0	50	62	13	50	13	50
	<i>E. durans</i>	15	13	53	80	47	0	47	33	27	47	13	53
	<i>E. hirea</i>	9	22	78	100	56	0	44	67	56	78	11	78
	All isolates	66	23	63	89	43	4	49	55	33	33	12	53
B	<i>E. faecium</i>	30	27	47	33	53	7	77	33	30	87	30	83
	<i>E. faecalis</i>	7	86	71	86	71	0	57	71	86	86	43	88
	<i>E. durans</i>	15	7	33	60	20	0	53	33	7	60	7	53
	<i>E. hirea</i>	8	25	50	75	63	13	75	13	38	88	0	75
	All isolates	60	36	50	76	52	5	66	38	40	80	20	74
C	<i>E. faecium</i>	33	12	36	76	42	3	36	30	15	42	0	48
	<i>E. faecalis</i>	8	63	50	75	50	0	13	38	38	13	13	50
	<i>E. durans</i>	20	25	65	70	55	0	25	30	10	40	15	50
	<i>E. hirea</i>	6	67	50	67	50	0	80	100	84	100	17	100
	All isolates	67	41	50	72	49	1	39	49	37	49	11	62
D	<i>E. faecium</i>	19	32	32	89	63	0	26	21	37	53	16	47
	<i>E. faecalis</i>	4	0	75	100	100	0	0	0	0	25	0	75
	<i>E. durans</i>	13	62	46	77	54	8	15	46	38	54	8	31
	<i>E. hirea</i>	5	20	20	100	40	0	20	40	0	40	20	40
	All isolates	41	28	43	92	64	2	15	27	19	43	11	48

Dox doxacycline, Amo amoxicillin, Pen penicillin G, Amp ampicillin, Ami amikacin, Van vancomycin, Ery erythromycin, Str streptomycin, CLi clindamicin, SXT sulfathiazole trimethoprim, Cep cephalothin

**Table 3** MDR pattern in enterococci isolated from poultry (P) and cattle (C)

Species	Isolates	MDR isolates	No. of antibiotics resisted	Total no. of isolates	Dominant resistance pattern	
					No. of isolates	Resistance pattern
<i>E. faecium</i>	116	85	4	23	7	Amo/Amp/Cep/Pen (P)
				8	3	Amo/Cli/Pen/Van (C)
			5	21	6	Amp/Cep/Cli/Pen/Van (P)
				8	4	Amo/Cli/Ery/Pen/Van (C)
			6	15	7	Amo/Amp/Cep/Cli/Pen/Van (P)
				10	8	Amo/Amp/Cli/Ery/Pen/Van (C)
			7	7	5	Amo/Amp/Cep/Cli/Ery/Pen/Van (C)
			10	5	5	Amo/Amp/Cep/Cli/Dox/Ery/Pen/Str/SXT/Van (P)
<i>E. faecalis</i>	27	22	4	5	3	Amo/Amp/Cep/Pen (P)
			5	4	2	Amo/Cli/Ery/Pen/Van (P)
	9	9				No dominant pattern (C)
<i>E. durans</i>	63	50	3	14	5	Cli/Ery/Str (P)
			4	9	3	Amo/Amp/Cep/Pen (P)
	21	21	5	10	4	Amo/Cli/Ery/Pen/Van(C)
<i>E. hirea</i>	28	26				No dominant pattern (P)

not observed. Only one isolate showed resistance to Gen/Str/Cip/Amp/Amo (Table 4).

## Discussion

Our finding showed that *E. faecium* was the dominant species in poultry, followed by *E. durans*, *E. hirea*, and *E. faecalis*. The absence of *E. hirea* in cattle in this study is distinctly different from the study in Kansas where *E. hirea* was the most abundant (Anderson et al. 2008).

Supplementing animal feed with antimicrobial agents to enhance growth has been a common practice for more than 30 years and is estimated to constitute more than half the total antimicrobial use worldwide (Mathew et al. 2007). The use of antibiotics for gaining these benefits selects resistant bacteria in the farms. In our study, the magnitude of resistance to different antimicrobials was distributed across all *Enterococcus* spp. isolated from poultry. The development of resistance to most antibiotics with different modes of action is considered to be a serious public health concern and the problem becomes more severe in developing counties like Ethiopia.

About 50% or more of our *E. faecium* isolates were resistant to six different drugs, and resistance to Cli and Pen was higher than that reported from Japan (Yoshimura et al. 2004) and USA (Hayes et al. 2004), respectively, but resistance to Amp was lower than that reported from Botswana (Chingwaru et al. 2003). Over 50% of *E. faecalis* isolates were resistant to Amp and this frequency is higher than that reported from Japan and USA (Yoshimura et al. 2004; Hayes et al. 2004).

Vancomycin-resistant enterococci (VRE) are an emerging international threat to public health all over the world. Van resistance in our study was higher than that reported from USA and Australia (Consumers 2003), New Zealand (Manson et al. 2004), and Korea (Seong et al. 2004). However, this figure is far less than the report from Sweden (79%; Sahlström et al. 2009). Delayed resistance to Van was detected in a few *Enterococcus* isolates from animal feces obtained from five European countries (de Jong et al. 2009). The occurrence of Van resistance in 44% of poultry isolates and 17.2% of cattle dung isolates in our study was markedly higher than in those isolates from clinical specimen in Iran (4.38%; Akhi et al. 2009) and hospitalized patients, out-patients, and healthcare

**Table 4** Drug resistance pattern in *Salmonella* isolates from poultry (P) and cattle(C)

No. of antimicrobial resisted	Number of isolates	Antimicrobial resistance pattern
1	18	Cep(1; P) Str (10; 9P, 1C) Amo(3; 1P, 2C, Kan(1; P) Gen(3; P)
2	17	Amo/Str (2; P) Gen/Str(9; 8P,1C) Amp/Str (1; P) Amp/Gen(1; P) Kan/Str(3; P) Amp/Amo(1; P)
3	9	Amo/Amp/Cep(1; P) Amo/Kan/Str (2; P) Amo/Amp/Str(2; P) Cep/Gen/Str(1; P) Amp/Gen/Str (3; P)
4	4	Amo/Amp/Cep/Str (2; P) Amo/Amp/Kan/Str (1; P) Amp/Cep/Gen/Str (1; P)
5	2	Amo/Amp/Cep/Gen/Str (1; P) Amo/Amp/Cip/Gen/Str (1; C)
6	2	Amo/Amp/Cep/Cip/Gen/Str/(2; P)

workers from Ethiopia where no Van resistant isolate was found (Worku 2005). This would indicate that, although Van resistance was not detected from human specimen, its wide distribution in poultry and cattle population is a public health threat, as the resistant strains could easily find their way in the human food chain. *E. faecalis* and *E. faecium* are the most common causative agents of nosocomial infections and our isolates from cattle and poultry had developed resistance to Vancomycin, which is the last drug of choice for infection caused by these species. Risk assessment studies have shown that chicken contributed more risk than other animal products in transmitting resistant enterococci to humans (Presi et al. 2009). However, recent pulsed field gel electrophoresis studies showed that transmission of resistant enterococci from animal sources to humans or vice versa were less common (Katsunuma et al. 2008).

Multiple resistance of enterococcal isolates in our study to three or more drugs was higher than the isolates from USA and Australia (Wallinga et al. 2002; Consumers 2003.) Since use of antibiotics in the farm environment in Ethiopia is not widespread, the resistance genes might be imported from abroad with the live chicks where antibiotic use is part of modern agriculture. The dissemination of resistance may be favored by the poor farm management practice in Ethiopia. We isolated VRE from the four farms considered in the study, but the proportion of the isolates that showed resistance varied among farms. Farm A (50% VRE isolates) and farm B (36.7% VRE isolates) commonly imported breeder (parent stock) and sometimes day-old chicks. In the process, resistance bacteria and genes might also be imported. These farms multiply and distribute chickens to other farms, small-scale producers, and directly to farmers or via Bureau of Agricultures and non-governmental organizations.

The proportion of multiple drug resistant isolates in our study was higher than the isolates from hospitalized patients, outpatients, and healthcare workers from Ethiopia (Worku 2005). This widespread dissemination of MDR isolates in food animals (cattle and poultry) may, thus, be a possible risk for acquisition of MDR by humans.

In the present study, resistance to eight antibiotics was observed in all the three enterococcal species isolated from cattle. The development of resistance to these drugs especially in *E. faecium* and *E. faecalis*, which are known to cause opportunistic infections in humans, is a point of concern. If these isolates pass through the food chain and establish in humans to cause infections, the treatment options become limited and, are thus, considered a serious public health threat.

Prevalence of *Salmonella* in poultry considered in our study was higher than that reported from Belgium (Van Hoorebeke et al. 2008) and Germany where no *Salmonella* was isolated from broiler flocks (Pieskus et al. 2008). Our result, however, was comparable to that obtained from the Netherlands (Pieskus et al. 2008) and India (Murugkar et al. 2005), but lower than the rate observed in the USA (Hayes et al. 2004) and Botswana (Gaedirelwe and Sebunya 2008). The prevalence of *Salmonella* in cattle in our study was far less than the result obtained from healthy cattle in USA. (Oloya et al. 2007). The prevalence in poultry



was higher than that in cattle, possibly because of the high density stocking in poultry which may facilitate horizontal transmission of pathogen (Barbour and Nabbut 1982). In Ethiopia, cattle graze in the open and the chance for horizontal transmission of pathogens is limited.

The frequency of *Salmonella* isolates resistant to one or more antibiotics was much higher than that observed in US dairy cows (Lundin et al. 2008). This indicated the wider dissemination of resistant isolates both in intensive and conventional farming systems in Ethiopia.

Resistance of *Salmonella* to the commonly used antibiotics in human medicine such as that to Str, Amo, Amp, and Gen was observed in this study at higher frequency in those isolates from cattle and poultry. Results of the present study indicated the potential importance of cattle and chicken as a source of single and multiple antimicrobial resistant *Salmonella* isolates to commonly used antibiotics. Further detailed molecular studies are essential on the frequency, source of acquisition of resistant genes, and distribution of resistant pathogens, known or opportunistic, among food animals, food products, and humans in Ethiopia.

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