

ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

**DISTRIBUTION OF DRUG RESISTANCE AMONG ENTEROCOCCI,
SALMONELLA AND *ESCHERCHIA COLI* O157:H7 ISOLATES FROM
POULTRY AND CATTLE FAECES**

BY
BEHAILU BEKELE

MAY, 2006

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**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF THE
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TABLE OF CONTENTS

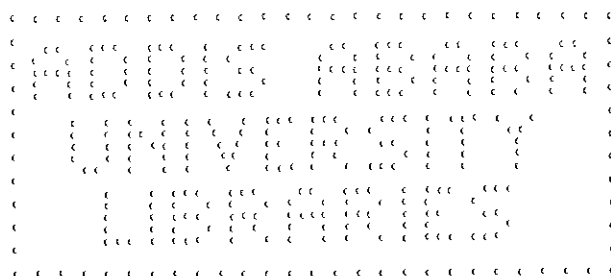
Content	Page
ACKNOWLEDGEMENT.....	I
TABLE OF CONTENTS.....	II
LIST OF TABLES.....	VI
LIST OF FIGURES.....	VII
LIST OF ABBREVIATIONS AND SYMBOLS.....	VIII
ABSTRACT.....	X
1. INTRODUCTION.....	1
2. LITERATURE REVIEW.....	4
2.1. Enterococci.....	4
Taxonomy.....	4
Habitat.....	4
Isolation and characterization.....	5
Pathogenicity and virulence.....	7
Antibiotic resistance.....	8
Applications of enterococci.....	9
2.2. <i>Escherichia coli</i> O157:H7.....	10
Ecology	10
Pathogenicity and virulence.....	10
Prevalence and drug resistance pattern.....	12
2.3. <i>Salmonella</i>	13
General characteristics.....	13
2.4. Antimicrobial use in farm animals.....	14
Growth promotion.....	15
Therapy, control and prevention.....	16

2.5. Transmission of drug-resistant bacteria from animals to	
Humans.....	17
3. MATERIAL AND METHODS.....	20
3.1. Collections of samples.....	20
3.1.1. Poultry.....	20
3.1.2. Cattle.....	20
3.2. Isolation of enterococci.....	20
3.3. Characterizations of enterococci.....	20
3.3.1. Microscopic examination.....	21
3.3.2. Catalase test.....	21
3.3.3. Gram reaction (KOH test).....	21
3.3.4. Growth in 6.5% NaCl.....	21
3.3.5. Growth at 45°C.....	21
3.3.6. Test for carbohydrate fermentation.....	21
3.4. Isolation and characterization of <i>Salmonella</i>.....	22
3.4.1. Primary enrichment (pre-enrichment).....	22
3.4.2. Secondary enrichment.....	22
3.4.3. Solid media.....	22
3.4.4. Biochemical identifications.....	23
3.4.4.1. Triple sugar Iron Agar (TSIA) (Oxoid).....	23
3.4.4.2. Lysine Iron Agar (Oxoid).....	23
3.4.4.3. Urea Agar (Oxoid).....	23
3.4.4.4. Simmons Citrate Agar (Oxoid).....	23
3.4.4.5. SIM Medium (Oxoid).....	24
3.4.4.6. Mannitol broth (1%).....	24
3.4.4.7. Sucrose broth (1%).....	24
3.4.4.8. Glucose broth.....	24
3.5. Isolation of <i>E. coli</i> 0157:H7.....	24
3.5.1. Sorbitol Mackonkey agar (SMAC).....	24
3.5.2. Microscopy and KOH test	25
3.5.3. Indole test.....	25

3.5.4. Latex agglutination test.....	25
3.6. Drug susceptibility testing.....	25
3.6.1. Enterococci.....	25
3.6.2. <i>Salmonella</i>	26
3.7. Data analysis.....	26
4. RESULTS.....	27
4.1. Prevalence and distribution.....	27
4.1.1. Enterococci from poultry.....	27
4.1.2. Enterococci from cattle.....	27
4.1.3. <i>Salmonella</i>	28
4.1.4. <i>Escherichia coli</i> O157:H7.....	28
4.2. Antimicrobial resistance.....	28
4.2.1. Enterococci from poultry.....	28
4.2.2. Enterococci from cattle.....	32
4.2.3. <i>Salmonella</i>	35
5. DISCUSSION	37
6. CONCLUSION AND RECOMMENDATIONS.....	46
7. REFERENCES.....	48

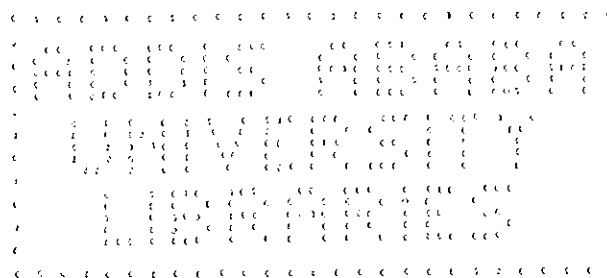
LIST OF TABLES

	Pages
Table 1. Percentage distribution of enterococcal isolates in the four farms.....	27
Table 2. Percentage of resistance in the four enterococcal species from poultry.....	28
Table 3. Proportion of resistant enterococcal isolates in the four farms.....	29
Table 4. Percentage of isolates resistant to antibiotics by farm and species with P-values from the analysis of variance.....	31
Table 5. MDR pattern in enterococci isolated from poultry.....	32
Table 6. Proportion of enterococcal isolates from cattle resistant to the different antibiotics tested.....	33
Table 7. MDR pattern in enterococci isolated from cattle.....	34
Table 8. MDR pattern in <i>Salmonella</i> isolated from poultry.....	35



LIST OF FIGURES

	Page
Fig 1. Differentiation of enterococci from other gram-positive, catalase – negative cocci.....	6
Fig 2. Some routes of transmission for antibiotic resistant gastrointestinal Pathogens or normal intestinal flora between animals and humans.....	19
Fig 3. Number of enterococcal isolates from poultry showing multiple drug resistance.....	30
Fig. 4. MDR in enterococcal isolates from cattle.....	34
Fig.5. <i>Salmonella</i> isolates from poultry resistant to the antibiotics tested.....	35
Fig. 6. <i>Salmonella</i> Isolates from cattle that showed resistance to the different antibiotics.....	36



LIST OF ABBREVIATIONS AND SYMBOLS

AGP	Antimicrobial Growth Promoters
BEA	Bile Aesculin Agar
BPW	Buffered Peptone Water
CATA	Citrate Azide Tween Agar
CDC	Centers for Disease Control and Prevention
DAEC	Diffusely Adherent <i>E.coli</i>
EAEC	Enteroadherent <i>E.coli</i>
EAggEC	Enteragggregative <i>E.coli</i>
EEC	Enterovirulent <i>E.coli</i>
EHEC	Enterohemorrhagic <i>E.coli</i>
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EIEC	Enteroinvasive <i>E.coli</i>
EPEC	Enteropathogenic <i>E.coli</i>
ETEC	Enterotoxigenic <i>E.coli</i>
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GDP	Gross Domestic Product
GRE	Glycopeptide Resistant Enterococci
HC	Hemorrhagic colitis
HUS	Hemolytic Uremic Syndrome
KAA	Kanamycin Aesculin Azide Agar
LEE	Locus of Enterocyte Effacement
MDR	Multiple drug resistance
NCCLS	National Committee for Clinical Laboratory Standards
STEC	Shiga-like Toxin producing <i>E. coli</i>
TTP	Thrombotic Thrombocytopenic Purpura
USDA	U.S. Department of Agriculture
VRE	Vancomycin Resistant Enterococci

VTEC

Verotoxin Producing *E. coli*

WHO

World Health Organization

ABSTRACT

Surveillance of the prevalence of zoonotic bacteria and resistant commensals in food animals is necessary to control the spread of the pathogens and resistant commensal bacteria to humans through contaminated animal food products and to the environment via their faecal material. This study was designed to see the prevalence of antibiotic resistance in enterococci from poultry and cattle; to assess the prevalence of *E.coli* O157 H7 from cattle, and that of *Salmonella* from cattle and poultry. Two hundred eighty cloacal swabs collected from poultry were examined for the presence of *Salmonella* and enterococci and the isolates were tested for their resistance to an array of 8 and 11 different antibiotics, respectively. Similarly, 450 fresh faecal samples collected from cattle were examined for the presence of *E. coli* O157 H7, *Salmonella* and enterococci. The *Salmonella* and enterococci isolates from cattle were tested for their resistance to an array of 8 and 9 different antibiotics, respectively. *Enterococcus faecium* was the most dominant species in both poultry and cattle (49.6% and 34/64), followed by *Enterococcus durans* (26.9% and 21/64). The prevalence of *Salmonella* in poultry and cattle was 15.4% and 1.8%, respectively. None of the cattle faecal samples was positive for *Escherichia coli* O157:H7. Resistance to the antibiotics tested was seen in all species from poultry. All *E. faecalis* isolates showed susceptibility to amikacin. There was no significant difference among farms and species in their percentage of resistant isolates to the different antibiotics, except difference among farms in percentage mean resistant isolate for penicillin G and vancomycin (at $\alpha=0.05$). Vancomycin resistance was observed in 44% of enterococcal isolates from poultry in the four farms and in 17% isolated from cattle. Multiple drug resistance (MDR) was observed in 78.2% and 90.6% enterococcal isolates from poultry and cattle, respectively. Enterococcal isolates from cattle showed resistance with varying percentage to all antibiotics tested, except amikacin. Among the *Salmonella* isolates 16 from poultry and 1 from cattle showed MDR. The presence of MDR isolates in the commensal and pathogenic microbes is a serious public health concern. The government and concerned bodies should focus and work more in the area.

Key words/phrase: Antibiotic resistance, Enterococci, *Escherichia coli* O157:H7, MDR, *Salmonella*

1. INTRODUCTION

The global demand for food is increasing because of the growing world population. At the same time, availability of arable land is shrinking. Animal productions have made and will continue to make important contribution towards meeting the need for more food. In many areas of the world, however, there is a problem in the safety of the foods.

Ethiopia is a country with agricultural economic base. More than 85% of the population is dependant on agriculture. Livestock production plays an important role in Ethiopia's economy. Estimates for 1987 indicated that livestock production contributed to one-third of agriculture's share of GDP (Gross Domestic Product), or nearly 15 percent of total GDP (WWW. photius. com/countries/ethiopia/economy). 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 (Alemayehu Mengistu, 2005). Larger segment of the rural and urban population is dependant on livestock for food and generation of income. Thus, many zoonotic bacterial pathogens can reach humans through consumption of contaminated foods and food products from these animals and through close contact with these animals.

Antibiotics are substances (sometimes termed as antimicrobials), including synthetic and semi synthetic ones that are active against microbes and that are used for the prevention and treatment of bacterial infections in humans and animals (Torrence, 2001; Phillips *et al.*, 2004). Antibiotics were once called miracle drugs because they revolutionized treatment of disease, curing bacterial infections that used to lead to debilitation and often to death. Not only humans, but also animals benefited from these wonder drugs. Nevertheless, over the years, some bacterial pathogens have developed resistance to the antibiotics that once spelled their doom. Antibiotic resistance is an ability of bacterial population to survive the effect of inhibitory concentration of antimicrobial agents (Davison *et al.*, 2000; Lukasova and Sustackova, 2003).

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 antimicrobial agents used in animal care are also important, not only in increasing the resistance in animal pathogens, but also in bacteria transmitted from animals to humans (WHO, 1997).

The exposure of bacterial flora in food animals to different antibiotics has favored the selection and accumulation of antibiotic resistant organisms. The ability of these bacterial strains to reach humans may have a major impact on the degree of success in treating infectious diseases in man. The increase in the prevalence of antibiotic resistance bacteria has compounded the incidence and danger of new and reemerging infections.

The use of antibiotics for agricultural purpose is part of modern agriculture. In developed countries like USA, more than 1 million tons of antibiotic are released to the biosphere during the past fifty years, out of which around 50% are estimated to flow in to the veterinary and agricultural channel (Davinson *et al.*, 2000). Some antibiotic are used both in the veterinary and human medicine. Penicillin, polymyxin, cephalosporin, tetracycline, spectinomycin, chloramphenicol, lincosamide, trimethoprim, and quinolones are few among those.

The veterinary use of antibiotics includes their use in farm animals. They are used in the therapy, prophylaxis, and to increase growth and feed efficiencies. The frequently exposed groups of microbes during the use of antibiotics for all these purposes are the enteric ones hence there is a possible development of resistance in the pathogenic and commensal bacteria. The commensal bacteria constitute a reservoir of resistant genes for the pathogenic bacteria. Their level of resistance is considered as a good indicator for the selection pressure of antibiotic use and for resistance problem to be expected in pathogens (WHO, 2000).

Microbial resistance to antibiotics is a worldwide problem. It can cause environmental problem. Before the widespread use of antibiotics, resistant strains were a small fraction of the microorganism's ecosystem. Resistant strains of both harmful and harmless bacteria are replacing antibiotic susceptible bacteria. Resistant bacteria in one environment may not be confined to that specific environment, but can be carried thousands of miles away by wind, water, animals, food, or people. The ubiquitous presence of resistant microorganisms has upset the delicate balance of microorganisms in the environment (WHO, 2003).

The other reason for antibiotic resistance to be of concern is its economic impact. The impact may be seen from different perspective: due to infection by resistant bacterial strains, patients incur added cost for drug, services and laboratory tests due to their prolonged stay in treatments. It also threatens the wish of drug

industries to maintain the life of their current antimicrobial drugs on the one hand, and it may make their product no more competitive, opening up the field for a product that may have been less marketable. It costs their customers more or their products become less safe or effective (McGowan, 2001; Howard *et al.*, 2003).

Agricultural antibiotic use has undesirable consequences (Smith *et al.*, 2002) such as the potential development of antimicrobial resistant zoonotic food borne bacterial pathogens and subsequent transmission to humans as food contaminants (White *et al.*, 2002).

The presence of reliable information about the prevalence and susceptibility of zoonotic pathogenic and commensal bacteria to different antibiotics is important to improve the quality of antibiotics used for treatment, to influence the pattern of antibiotic usage and to assist governments to formulate policy for the supply and use of antibiotics.

1.1. Objectives

1.1.1. General objective

The general objective of this study is primarily to assess the distribution of antibiotic resistance in enterococci isolated from the faeces of cattle and poultry and prevalence and antibiotic resistance pattern of *E. coli* O157:H7 in cattle and that of *Salmonella* in cattle and poultry

1.1.2. Specific objectives

The specific objectives of this study are

1. to isolate, characterize and test the resistance pattern of enterococci from the faeces of poultry in intensive poultry farms and in faeces of cattle.
2. to see the prevalence and the resistance pattern of *E.coli* O157:H7 from the faeces of cattle collected around Addis Ababa and some parts of the rift valley
3. to see the prevalence and antibiotic resistance pattern of *Salmonella* from poultry and cattle

2. LITERATURE REVIEW

2.1. Enterococci

Taxonomy

The genus *Enterococcus* was traditionally considered as part of the lactic acid bacteria. The classification of it as part of the group D streptococci dates back to the system established by Rebecca Lancefield in the early 1930s (Morrison *et al.*, 1997). It was Sherman in 1937 who divided streptococci in to four groups: enterococci (faecal streptococci), the diary streptococci (lactic), the viridians and the pyogenous streptococci group. 'Viridans' and 'Streptococci' have been later changed in to oral and faecal streptococci, respectively (Klein, 2003).

The first faecal organism was classified in the genus *Streptococcus* as *Sreptococcus faecalis* by Andrews and Horder in 1906 and the second was described as *Streptococcus faecium* by Oral-Jensen in 1919 (Morrison *et al.*, 1997). It was in 1984 that enterococci have been given a formal status after DNA-DNA and DNA-RNA hybridization studies demonstrated their more distant relationship with streptococci (Klein, 2003) but the term enterococci was first used by Thiercelin in 1899 to describe a Gram positive diplococcus that was of intestinal origin, that formed pairs and short chains (Morrison *et al.*, 1997). The original description of *Enterococcus faecium* and *Enterococcus faecalis* was given by Schleifer and Kilpper Balz in 1984. Since then, a variety of species have been described within the genus and presently more than 20 species have been included (Giraffa, 2003). But faecal enterococci include four species; *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus durans* and *Enterococcus hirae* (Tejedor *et al.*, 2001).

Habitat

Enterococci are normally found in the intestine of nearly all animals, the lower gastrointestinal tract of animals is considered to be their natural habitat but they can also be able to colonize a variety of other sites, including the upper gastrointestinal tract, the lower and upper genital tract and the oral cavity (Morrison *et al.*, 1997).

A certain species often predominates in a specific host; *Enterococcus faecalis* predominates in cattle, pigs, dogs, cats, horses, goats and rodents (Morrison *et al.*, 1997). *E. faecalis* and *E. faecium* are the most

frequent species in the human intestine (Rince *et al.*, 2003), the later is the most frequent species in the intestine of poultry and sheep and *Enterococcus durans* occurs less frequently in poultry and sheep (Klein, 2003). Enterococci are ubiquitous and can be found free-living in soil, on plants, or in dairy products, enter milk and meat products due to contamination during slaughtering and milking; and vegetables can also be contaminated due to the use of animal excrement as fertilizer. They can also be recovered from water and serve as indicator of faecal contamination (Kayser, 2003; Harwood *et al.*, 2004).

Their exceedingly hardy natures namely ability to grow at different temperatures ranging from 10°C to 45°C, ability to survive in hypotonic, hypertonic, acidic, or alkaline environments, contribute to their existence in different environments (Hugas *et al.*, 2003). Their ability to tolerate, and grow in the presence of, sodium azide and concentrated bile salts that inhibit or kill other microorganisms is used as a selective factor in agar-based media (Huycke *et al.*, 1998a).

Isolation and characterization

Due to the importance of enterococci in different foods, feeds, clinical and environmental samples, a diversity of media were developed for their isolation. More than 100 modifications of selective media are in use for the isolation of enterococci from various specimens. It is not possible to recommend a universal medium that meets all requirements. The choice of a particular medium depends on whether enterococci are to be counted in total, or whether the source of the specimen is highly contaminated or not (Domig *et al.*, 2003).

Different media have been developed to isolate enterococci from the intestine of animals and their faeces. Citrate azide tween agar (CATC), Kanamycin aesculin azide agar (KAA), Bile aesculin azide agar (BEA) and different modifications of these media can be used (Morrison *et al.*, 1997). In samples like faeces that contained mixed flora, variation of selective parameters and compounds can be used to isolate enterococci and to reduce the growth of the background microflora. Moreover, enterococci cannot grow rapidly because of their requirement for several vitamins and amino acids (Klein, 2003).

The use of elevated incubation temperatures (42-45°C) and the duration of incubation (18 or 24 hours) may influence the selectivity of some media. Selective additives such as sodium azide, potassium thiocyanate, dyes as crystal violet or antibiotics like kanamycin and gentamicin can also be used (Domig *et al.*, 2003).

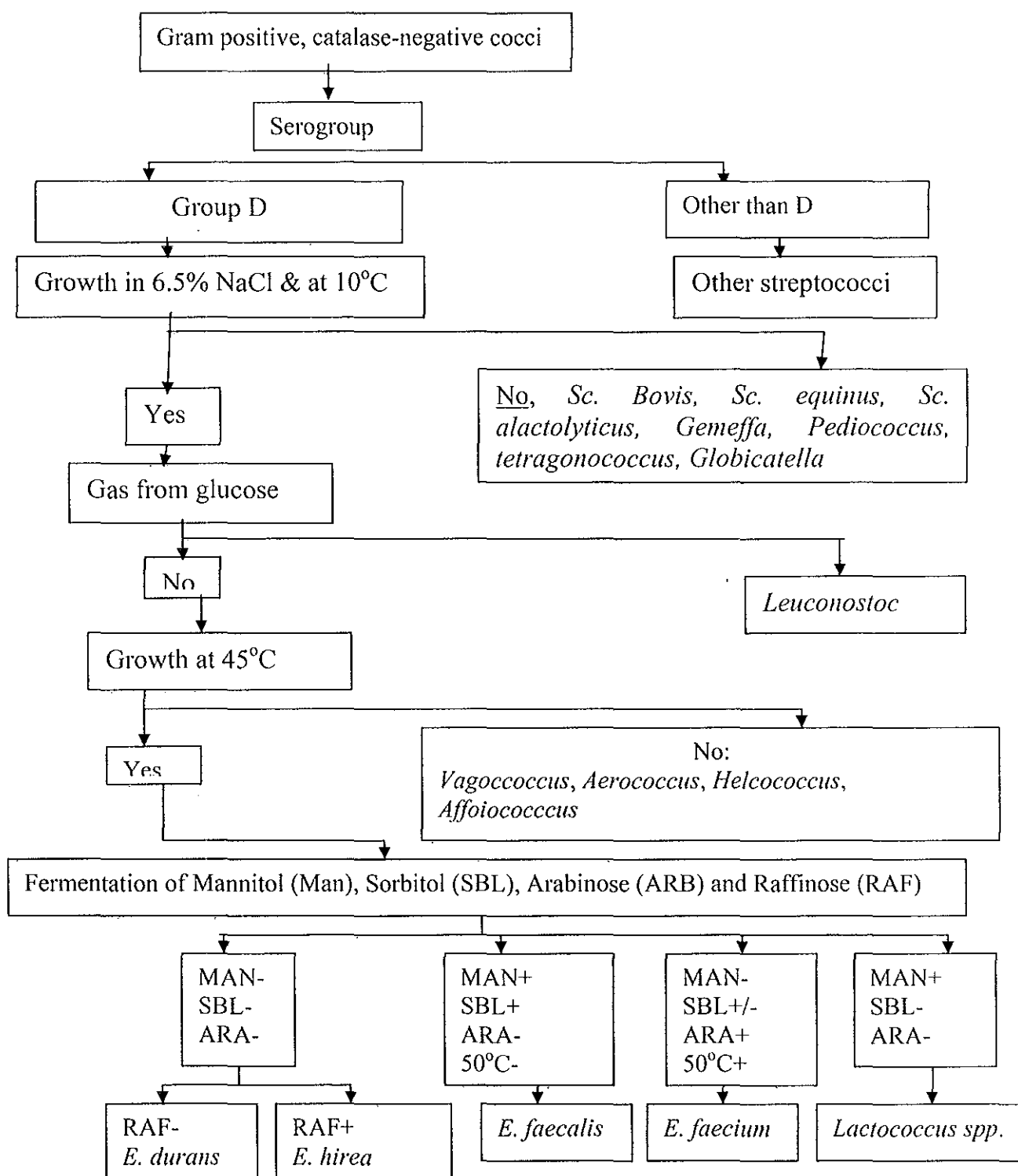


Fig 1. Differentiation of enterococci from other Gram-positive, catalase-negative cocci (taken from Klein, 2003)

None of the isolation media are specific and the isolation of small false positives necessitates using additional confirmatory tests. During the identification process, the primary work is to differentiate enterococci from other Gram positive, catalase negative cocci. Although few species like *Streptococcus bovis* and *Streptococcus equinus* belong to serogroup D, differentiation from the genus *Streptococcus* can be made by confirming the serological group D for enterococci. Different fermentation properties, enzymatic characteristics and growth at a defined temperature can be used to differentiate the genus into species and other cocci, that are able to grow together, such as *Pediococcus* and *Lactococcus* (Manero and Blanch, 1999).

Pathogenicity and virulence

Enterococci exist as normal human commensals (Harwood *et al.*, 2004). Their pathogenicity was addressed for the first time at the end of the 19th century by MacCallum and Hastings after isolation from a case of acute endocarditis (Mundy *et al.*, 2000). They have a particular medical relevance in hospitalized patients and immuno-compromised individuals. Their increased incidence as nosocomial pathogen is compounded by their multiple antibiotic resistance (Cocconcelli *et al.*, 2003). They take the third place of bacterial pathogen associated with nosocomial infection, after staphylococci and *Escherichia coli* (Peters *et al.*, 2003). It has been well documented that enterococci are etiological agents of endocarditis, urinary tract infections, bacteremias, wound, and intra-abdominal infections (Huycke *et al.*, 1998b). Approximately 85 to 90% of enterococcal infections are attributed to *Enterococcus faecalis* and 5 to 10% are attributed to *Enterococcus faecium*. Infections caused by other *Enterococcus* species occasionally emerge and have warranted attention (Simjee *et al.*, 2002).

Little is known about the factors contributing to the pathogenesis of enterococci. The major determinants of virulence, known so far to play some role in their pathogenesis, are certain secreted products on the one hand and adherence factors on the other. Studies on *E. faecium* have shown the role of antibiotic resistance for the pathogenesis of enterococci (Mundy *et al.*, 2000).

Bacterial adherence to the host tissue is the first step in the infection process. For gastrointestinal commensals like enterococci, there has to be a close association between the bacteria and the host otherwise the organism would be eliminated due to normal intestinal motility. Specific means of attachment has to be there. Adhesins, called enterococcal surface proteins (ESP) and gelatinase (Gel), which is an

extracellular metalloendopeptidase that promote binding to intestinal mucosa and extra cellular surface proteins, are assumed to be responsible for adherence and colonization (Franz *et al.*, 2001).

Cytolysin is considered as the most important virulence trait in *Enterococcus faecalis*. It is a unique bacterial toxin and is a family of small, post-translationally modified peptides. It is located in the transmissible plasmids and occasionally in the chromosome. Cytolysin causes rupture of target membranes of bacterial cells, erythrocytes and eukaryotes (Dupont *et al.*, 1998). It occurs at a frequency of 45-60% in clinical enterococcal isolates. An experiment in an animal model has shown a five-fold increased risk of death for cytolytic strains as compared with the non-cytolytic strains (Huycke *et al.*, 1998b).

Another trait suggested to contribute to the pathogenicity of enterococci that is common in *E. faecalis* isolates from surgical and neurosurgical intensive care patients is protease. Studies have shown that around 54% of the enterococci isolated from infected patients produced this enzyme as compared to only 12% and 14% of the isolates from non-infected hospitalized patients and healthy volunteers, respectively. This suggests the contribution of protease for the virulence of enterococci (Kayser, 2003).

Antibiotic resistance

The prevalence and degree of antibiotic resistance found in indicator bacteria is considered to be a good signal for the selective pressure of antibiotic usage and the resistance in these bacteria can be considered as an early warning for resistance to be expected in potential pathogenic bacteria (Van den bogaard *et al.*, 2000).

Natural and acquired antibiotic resistance is characteristics of enterococci (Kayser, 2003). Ampicillin and aminoglycosides have been considered as drugs of choice for the treatment of serious enterococcal infections but resistance to these drugs becomes common now-a-days (Klein *et al.*, 1998). The resistance of enterococci isolated from poultry to different antibiotics has a worldwide phenomenon. Studies conducted in China, Japan and Brazil showed high level of gentamicin resistance in *Enterococcus* isolated from broiler chickens (Harada *et al.*, 2004). A study carried out to asses the susceptibility profile of 541 enterococcal isolates from 81 poultry farms in eastern Seaboard in the USA, to antimicrobial agents used in the production and clinical environment, showed 63% of the *E. faecium* isolates were resistant to

streptogramin quinupristin-dalfopristin and 7% of *E. faecalis* isolates were resistant to gentamicin (Hayes *et al.*, 2004). Among vancomycin resistant *E. faecium* isolates from Italian poultry, 48.9% showed multiple resistances (Busani *et al.*, 2004).

Drug resistance is a worldwide problem. Enterococcal isolates from foods of animal origin in different parts of the world showed resistance to antibiotics used for the treatment of humans and animals. These isolates often carry transferable resistance genes mediating resistance to the antibiotics used in human medicine. The potential impact of this resistant gene pool on human health has caused much public health concern (European commission, 1999).

Applications of enterococci

Enterococci can be used in many different applications. They can be used as dairy starter cultures. They occur as non-starter microflora in a variety of cheeses and play an acknowledged role in the development of organoleptic characteristics during ripening of many cheeses. The positive influence of *Enterococcus* on cheese seems due to specific biochemical traits such as lipolytic activity, citrate utilization, and production of aromatic volatile compounds. Some enterococci of dairy origin have also been reported to produce bacteriocins (enterocins) inhibitory against food spoilage or pathogenic bacteria, such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio cholerae*, *Clostridium* spp., and *Bacillus* spp. They can also be used as starter cultures such as silage inoculants (Giraffa, 2003).

Some enterococcal strains have been used successfully as human probiotics. Probiotics are live microorganisms that, when ingested, have beneficial effect in the prevention and treatment of a disease. Their success as probiotics has been attributed to factors such as acid and bile resistance, bile salt hydrolase activity, production of antimicrobials and their ability to survive and compete in the gastrointestinal tract (Murry *et al.*, 2004). They contribute to the improvement of microbial balance and treatment of gastroenteritis in humans and animals (Hugas *et al.*, 2003). They play a role in flavor development and quality of cheese. Their use for these purposes may enable them to reach the gut and, if they are resistant to drugs, they may transfer resistance genes to other enteric pathogens.

2.2. *Escherichia coli* O157:H7

Ecology

Ruminants (cattle and sheep) are natural reservoirs of *E. coli* O157:H7 and it can also be isolated from deer, horses, pigs, dogs, and birds (Keskimaki *et al.*, 1998; Kudva *et al.*, 1998). This bacterial serotype is found as avirulent in the reservoir animals but the reason for this is not yet known. It plays a significant role in the epidemiology of human infections (Meyer-Broseta *et al.*, 2001; Omisakin *et al.*, 2003).

Many outbreaks associated with *E. coli* O157:H7 are associated with foods from these animals due to contamination during slaughtering, processing, or faecal contamination of water and vegetables by these animals. The other reported sources for this bacterial serotype include raw milk (Wang and Doyle, 1998), unpasteurized apple cider (Penner, 1995), water (drinking and swimming) (Keene *et al.*, 1994), vegetables; lettuce, radish sprout and alfalfa sprout (Beuchat, 1999). Direct contacts (animal-to-person or person-to-person) are also reported as causes of infection (Kudva *et al.*, 1998).

Different environmental parameters determine the growth and survival of microbes. *E. coli* O157:H7 has an optimal growth temperature between 30°C – 40°C and their growth is poor or absent at 44 °C - 46.5°C (Buchanan and Doyle, 1997). The organism was found to survive for more than a year when ovine faeces was used to make manure. In aerated ovine and bovine manure piles, it survived 4 months and 47 days, respectively (Kudva *et al.*, 1998). The ability of the pathogen to survive in the environment for a long period under different environmental conditions can be a possible risk for contaminating foods of plant origin and animal feeds that subsequently reach to humans.

Pathogenicity and virulence

Escherichia coli were commensal bacteria until they were discovered as important pathogen by Dr. Theodor in 1885 (Bell, 2002). From that time onwards some serotypes have become common to cause disease in humans and these types of *E. coli* are generally designated as enterovirulent *E. coli* (EEC). They can be divided into six sub groups: Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroadherent *E. coli* (EAEC) or enteroaggregative *E. coli* (EAggEC), and diffusely adherent *E. coli* (DAEC) (Chiueh *et al.*, 2001).

Enterohemorrhagic *E. coli* (EHEC) is the greatest microbial challenge at present. It has low infective dose, is infectious to humans, causes serious acute illness, is naturally occurring in cattle (and other animals) and it occurs globally (Bell, 2002). Since EHEC was reported for the first time, it has been given different names. Because of the production of cytotoxin on Vero cells in tissue culture, it was named Verotoxin producing *E. coli* (VTEC). Later it was discovered that the toxins are similar to shiga toxin, and then the organism was known to be shiga-like toxin producing *E. coli* (STEC) (Chiueh *et al.*, 2001).

Shiga-like toxin producing *E. coli* (STEC) are distributed world wide and are described as a major cause of food borne outbreaks in countries like the United States, Canada, Japan Europe, Argentina, Chile and Brazil. They contain many subgroups; one is EHEC. Enterohemorrhagic *E. coli* are classified in to more than 100 O:H serotypes which are associated with sporadic case of Hemolytic uremic syndrome (HUS). However, those belonging to O157 are the most prevalent causes of STEC diseases in humans (Paton *et al.*, 1996). *E. coli* O157:H7 is the predominant EHEC and was recognized first as a pathogen in 1982 in the USA when two outbreaks of severe bloody diarrhea in 47 individuals occurred in Oregon and Michigan in February and May, respectively. These happened after eating ground beef from the same fast food hamburger restaurant (Kudva *et al.*, 1998; White *et al.*, 2002).

Human infection due to this serotype can result in non bloody diarrhea, bloody diarrhea and more serious and potentially fatal complications such as hemorrhagic colitis (HC) (Kasrazadeh and Genigeorgis, 1995; White *et al.*, 2002). Hemorrhagic colitis is self limiting in healthy adults within four to ten days and 2-15% of the cases in children may develop into hemolytic uremic syndrome (HUS), that begins three to four days after the contaminated food is consumed (Penner, 1995). Hemolytic uremic syndrome is a complication due to the infection caused by *E.coli* O157:H7 (White *et al.*, 2002). It can be manifested by symptoms as acute abdominal cramps; bloody diarrhea; hemolytic anemia, a low grade fever; and urinary tract infection and thrombotic thrombocytopenic purpura (TTP), a disease of the central nervous system that causes seizures, coma, and blood clots in the brain (Penner, 1995).

Infections due to *E. coli* O157:H7 are common in the developed world. Studies by Centers for Disease Control and Prevention (CDC) showed that *E. coli* O157:H7 caused an average of 500 outbreaks that affect more than 73,000 persons and result in over 61 deaths each year in the United States (Feder *et al.*, 2004).

A critical element for its emergence as a food borne pathogen is the evolution of acid resistance, an attribute that promotes its survival in acid foods and results in efficient transmission with a low infective dose. The tolerance of highly acid environments is one aspect of overall durability, that is, its ability to remain viable in the external environment outside of the host (Whittam *et al.*, 1998).

Prevalence and drug resistance pattern

Cattle and sheep are thought to be reservoirs of *E. coli* O157:H7. A study in the USA has shown that the rate of prevalence is from 0.3% to 6.1% in cattle and the rate at which culture positive sheep samples occur is from 0.9% to 3.1% (Kudva *et al.*, 1998). The prevalence is variable with age of the animal: in cattle younger than 8 weeks (weaning age), it was less than 1.5% and in those animals between 8 weeks and 4 months it ranged from 1.8% to 5%. Adult dairy and beef cattle shed with a frequency of less than 0.7% (Meyer-Broseta *et al.*, 2001). The prevalence in food products of animal origin is variable among different countries. It was 1% in ewe's milk from Greece (Dontorou *et al.*, 2003), 6% in raw cow's milk from Egypt, 3% of milk tested from Australia and 0.3% from Germany. Similarly, its prevalence in meat products was 8.7% in hamburger from Colombia and 0.3% in minced meat sample from Denmark (Kudva *et al.*, 1998).

There were reports regarding isolation of *E. coli* O157 in Swaziland and South Africa from stool specimens from patients in diarrheal out break, from cattle dung specimens and from water (Effler *et al.*, 2001). Although few in number, it was also isolated from cockroaches (Erdaw Tachbele, 2004) collected from hospital and restaurants and from retail beef (Abraham Mikru, 2004) in Addis Ababa, Ethiopia. These were among the few reports in the developing world.

Currently antibiotics are not used to treat infections due to *E. coli* O157:H7 and they have been susceptible to many different antibiotics. Several studies recently demonstrated that antibiotic resistance was uncommon within this serotype from clinical isolates. One study has shown 5 out of 174 (2.9%) strains of *E. coli* O157:H7 demonstrated resistance to an antibiotic. According to the report from Center for disease control and Prevention (CDC), from 1983 to 1985, two out of 200 O157:H7 isolates became resistant at least to one antibiotic. Increased resistance to fosfomycin, which is the drug of choice for gastrointestinal infection due to *E. coli* O157:H7 had been reported (White *et al.*, 2002). The emergence of antibiotic resistance in this pathogen may be due to the use of antibiotics for growth promotion, for prophylaxis and treatment of infection in cattle. The threat is that the emergence of antibiotic resistance strains may allow

this pathogen to preferentially colonize cattle over other enteric bacteria leading to an increased prevalence in cattle. This could result in subsequently higher contamination of foods and the environment with this serotype.

2.3 *Salmonella*

General characteristics

Salmonella is a Gram-negative facultative rod shaped bacterium in the family Enterobacteriaceae. They are motile, oxidase negative and non-lactose fermenting bacteria. The genus currently includes more than 2400 serotypes (White *et al.*, 2002).

It lives in the intestinal tract of warm and cold-blooded animals but some species are ubiquitous and others are adapted to a particular host. *S. Typhi* and *S. Paratyphi A* are strictly human serovars that may cause severe disease associated with invasion of the blood stream. *S. Gallinarum*, *S. Abortusovis* and *S. Typhimurium* are respectively avian, bovine and porcine serovars. Some serovars (e.g *S. Typhimurium*) are ubiquitous (non-host adapted). Some species adapted to poultry are thought to cause fowl typhoid (Oliveira *et al.*, 2005).

This pathogen can be isolated from numerous animal species and it is known to be a principal zoonotic bacterium causing symptoms such as diarrhoea, fever and septicaemia. Its high morbidity and mortality rates make it one of the most economically significant pathogens in livestock production (Bischoff *et al.*, 2004). Swift treatment with appropriate antimicrobial agents remains economically important.

The clinical symptoms of acute disease in cattle by *Salmonella* are marked by fever, diarrhoeal stools, dullness, anorexia, and depressed production of milk. Infected animals consistently excrete large numbers of salmonellae in stools and may yield positive blood and milk samples in the febrile stage of illness. Abortion in cows is not uncommon and is generally associated with an infected fetus. High mortality (75%) in untreated animals occurs 4-7 days after the on-set of symptoms (Franco, 2004).

Salmonellosis in cows is an economically important bacterial diseases surpassed only by *E. coli*. The disease predominates in animals 2-6 weeks of age, with mortality rate of 10-60%. The acute clinical picture of the disease in calves includes bloody or mucoid stools, rapid dehydration, metabolic acidosis,

emaciation, and reduced growth rate (Daoust, 1989). *Salmonella* is also a causative pathogen of food-borne illness and remain a constant challenge to the feed and food industry throughout the world (Franco, 2004). There is considerable information on antimicrobial resistance in *Salmonella* of human and food animal origin and it has emerged as a considerable public health concern worldwide (Bayleyegn *et al.*, 2003a)

In humans, salmonellae are the causes of enteric fever (typhoid) resulting from bacterial invasion of the blood stream and acute gastroenteritis resulting from food borne infection (intoxication). Non-typhoidal salmonellosis in humans is usually a self-limiting disease confined to the gastrointestinal tract, but when infection spreads beyond the intestine or when immunocompromised persons are infected, appropriate antimicrobial treatment remains essential (Esaki *et al.*, 2004).

Through human or animal excretion, salmonellae are disseminated in the natural environment and can be found in water, soil, and plants used as food. The organism does not seem to multiply significantly in the natural environment (out of the digestive tract) but can survive several weeks in water and several years in soil if condition of temperature, humidity and pH are favorable (Bischoff *et al.*, 2004). Due to lack of hygienic handling of foods, *Salmonella* may be associated with all kinds of foods. Infection may occur due to ingestion of food that supports multiplication of *Salmonella*, as large numbers of ingested *Salmonella* are needed (WHO, 2005).

2.4. Antimicrobial use in farm animals

Microbial resistance to antibiotics is on the rise, in part because of inappropriate use of antibiotics in human medicine and because of practices in the agricultural industry. Intensive animal production involves giving animals large quantities of antibiotics to promote growth and prevent infection (Khachatourians, 1998).

Antimicrobial agents are usually used in agriculture as therapeutic agents against bacterial infections, as growth-promoting feed additives (Threlfall *et al.*, 2000) and prophylactic agents in animals (Lukasova and Sustackova, 2003). The inappropriate use of antibiotics in the agricultural setting for these purposes is a major contributor to the emergence of antibiotic-resistant bacteria (Khachatourians, 1998) and the

emergence of bacterial antimicrobial resistance is becoming a serious problem worldwide (White *et al.*, 2002).

Out of more than 1 million tons of antibiotics released in to the biosphere during the last fifty years, roughly 50% are estimated to have flown in to the veterinary and agricultural channel. The statistics obtained from the European Union of Animal Health for the year 1997 indicated 3,465,000 kg of antibiotics were used for animal health and 1,575,000 kg for animal growth promotion (Teuber, 2001). According to the data obtained from the union of concerned scientists, 35,000,000 pounds of antimicrobials are used annually out of which 13% were used in human medicine, 78% for non-therapeutic uses in agriculture and 6% for therapeutic use in agriculture (Shea, 2003).

Agricultural antibiotic use has undesirable consequences (Smith *et al.*, 2002) such as the potential development of antimicrobial resistant zoonotic food borne bacterial pathogens and subsequent transmission to humans as food contaminants (White *et al.*, 2002). Intensive farming units practice the use of antibiotics for different purposes; they represent a large reservoir of antibiotic resistant bacteria (Van den Bogaard *et al.*, 2000).

Growth promotion

Growth promotion is the administration of an antimicrobial, usually as a feed additive, over a period, to growing animals that results in improved physiological performance (Phillips *et al.*, 2004). Antimicrobial growth promoters (AGPs) are antibiotics added to the feed of food animals to enhance their growth rate and production performance. They are fed at low, generally at sub therapeutic concentrations (MCKellar, 1998). Antimicrobial growth promoters reduce normal intestinal flora (which compete with the host for nutrients) (Wegener *et al.*, 1999) and harmful gut bacteria (which may reduce performance by causing sub-clinical disease) (MCKellar, 1998). The effect on growth may be due to a combination of both fewer normal intestinal flora and fewer harmful bacteria (Wegener *et al.*, 1999).

Animals receiving antibiotics in their feed gain 4 to 5% more body weight than animals that do not receive antibiotics. More antibiotics are used in these manner than in medical applications. According to a study in Denmark in 1994, 24 kg of the glycopeptide vancomycin were used for human therapy, whereas 24,000 kg of the similar glycopeptide avopracin were used in animal feed. Similarly, Australia imported an average of 582 kg of vancomycin per year for medical purposes and 62,642 kg of avopracin per year for

animal husbandry from the year 1992 to 1996. Vancomycin and avopracin have the same mode of action; resistance to one can confer resistance to the other (Witte, 1998).

Antimicrobials approved by FDA (Food and Drug Administration) for growth promotion in farm animals are of two types; some like Amprolium, Arsanilic acid, Bambermycins, Carbadox, Laidlomycin, Lasalocid, Monensin, Roxarsone, Tiamulin are not identical or are chemically dissimilar to those drugs for human use. Others like Bacitracin, Chlortetracycline, Erythromycin, Lincomycin, Oxytetracycline, Penicillin, Sulfonamides, Virginiamycin and Tylosin are identical or chemically similar to human-use drugs (Shea, 2003)

Therapy, control and prevention:

According to the definitions given by The National Committee for Clinical Laboratory Standards (NCCLS) for herd antibiotic use, therapy is the administration of an antimicrobial to an animal, or group of animals, which exhibit frank clinical disease. Control is the administration of an antimicrobial to animals, usually as a herd or flock, in which morbidity and/or mortality have exceeded baseline norms. Prevention/prophylaxis is the administration of an antimicrobial to exposed healthy animals considered to be at risk, but before expected onset of disease and for which no etiological agent has yet been cultured (Phillips *et al.*, 2004).

Antibiotics, which are used for treatment or prophylaxis purposes usually are either broad or narrow in their spectrum of activity. A broad-spectrum antibiotic tends to be active against a broader range of bacteria including both Gram-negative and Gram-positive organisms while narrow spectrum antibiotics are active against either Gram positive or Gram negative. Broad-spectrum antibiotics tend to lead to the development of resistance in bacteria that are not the ones involved in the infection being treated. When antibiotic therapy is to be used, it should be targeted, as much as possible, to the pathogen, which means narrow-spectrum antibiotics should be chosen whenever possible (WHO, 1983a). Antibiotics should be used in the optimal dosages and regimens, and should be stopped when the infection is treated (Collignon, 2002).

In intensive poultry farms, when there is occurrence of poultry disease outbreak, producers treat all chicken, including those not infected, with antibiotics. These practices are also common in Ethiopia (personal communication). These would probably lead to the emergence of antibiotic resistance. Thus,

antibiotic treatment should be based on precise clinical diagnosis of the nature of the infective processes (WHO, 1983a). The use of antibiotics to animals for any purpose (growth promotion, prophylaxis, or therapy) leads to the accumulation of resistant bacteria in their flora. Antibiotics have been in use for all these purposes for many years and these activities contributed to the pool of resistant organisms in animals (WHO, 1983b).

The dangers of this pool are: antibiotic-resistant pathogens common to animals and man may reach man by cross-infection; antibiotic-resistant and non-pathogenic organisms in an animal may pass and colonize man, thereby carrying resistance plasmids (R plasmids) in to the human environment. These R plasmids may subsequently be transferred to human pathogens or to indigenous flora in the human body (WHO, 1983b).

Some of the antibiotics used for the treatment of infection in food animals are amoxicillin, bacitracin, cephalosporins, erythromycins, fluoroquinolones, gentamicin, lincomycin, neomycin, penicillin, streptomycin, tetracycline and sulfonamides (Shea, 2003), most of which are used for treating infections in human beings. Different workers have indicated that the use of different antimicrobial agents that have human analog (like avopracin and virginiamycin) which are analogs of vancomycin and quinupristin-dalfopristin, increased the likelihood of the development and spread of food borne pathogens of animal reservoir with cross resistance to antimicrobial agents used in human medicine (McDonald *et al.*, 1997). This has led the Danish government to ban the use of avopracin in 1995 and Virginiamycin in 1998; and the European Union to ban four growth promoters (avopracin, tylosin, bacitracin, and virginiamycin) in 1998 (Shea, 2003).

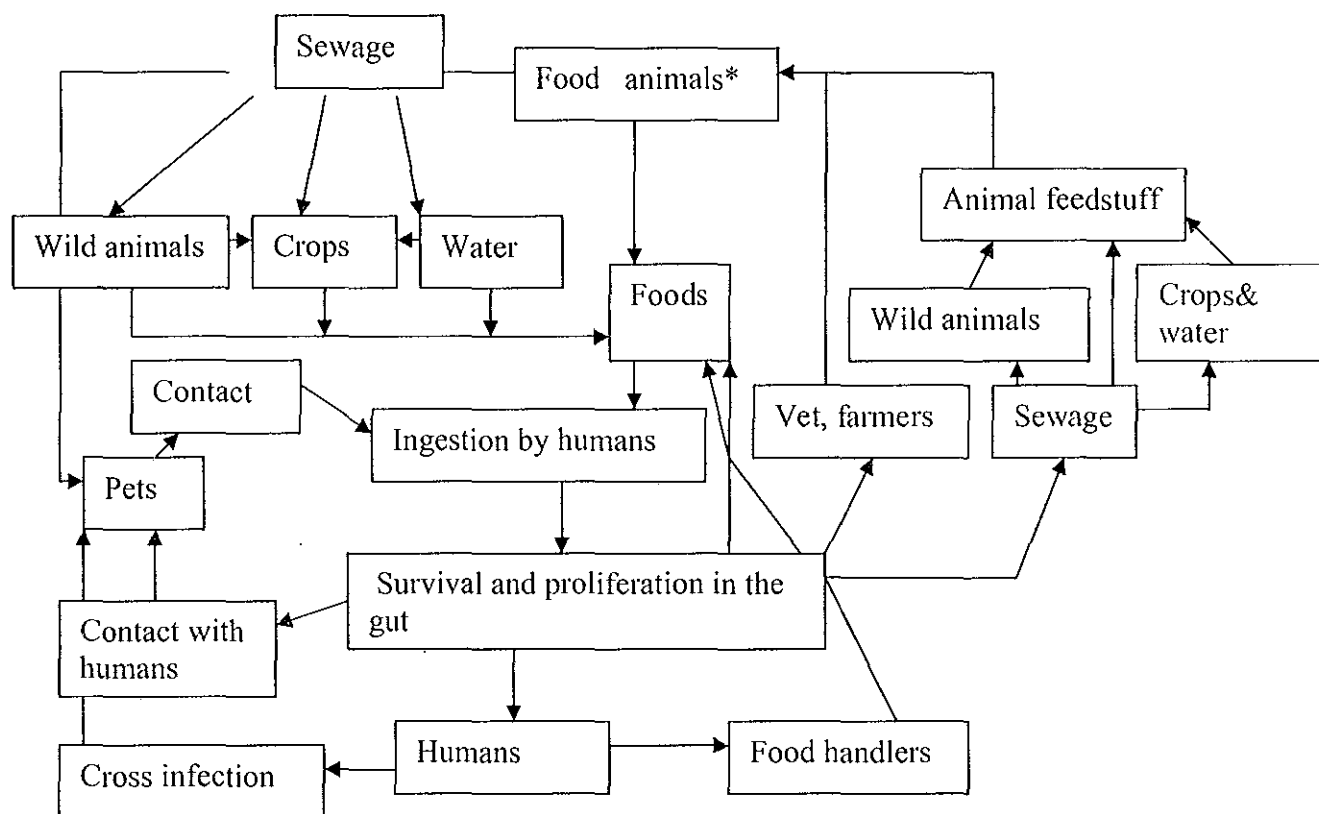
2.5. Transmission of drug-resistant bacteria from animals to humans

The prevalence and antibiotic resistance in a population are strongly correlated with antibiotic usage, as selection and dissemination of resistant bacteria are heavily augmented under selective pressure caused by antibiotics (Van den Bogaard *et al.*, 2000). The wide-spread use of antibiotics in animals for different purposes selects for resistance plasmids that are shared between animals and humans (Praff, 1990). There is a fear that the selection of antibiotic resistant commensal strains in the intestinal flora of food animals would lead to the increased pool of antibiotic resistance (WHO, 1983b). Later the resistance could be transferred to the pathogens in the gut of the animals, or they could contaminate foods of animal origin and transfer their resistance to other bacteria in the human gut (Van den bogaard *et al.*, 2000).

This resistance can reach the general environment via sewage. Wild animals, especially rodents, and birds, can acquire these environmental contaminants and pass them on, via their excreta, to grazing land or to the foodstuffs of food animals. Vegetables may also be contaminated from sewage, especially in countries in which human faeces is used as a fertilizer (Phillips *et al.*, 2004).

People can become infected by eating undercooked contaminated meat, or by eating other foods or using utensils that have been exposed to meat juices (Wallinga *et al.*, 2002). In addition, farmers, farm families, and slaughterhouse workers are routinely exposed to antibiotics or antibiotic-resistant bacteria, or both (Van den Bogaard *et al.*, 2000). Farm waste run-off can enter rivers, lakes, and ground water and these wastes are sometimes spread on agricultural fields as fertilizer as well. Because as much as 75% of an antibiotic in animal feed that passes undigested through the animal, wastes can contain antibiotics as well as antibiotic-resistant bacteria (Lipsitch *et al.*, 2002). That leads to the dissemination of drug resistant bacteria to the general environment.

Eating food that contains antibiotic-resistant pathogens can have a direct impact on health. Resistant strains of bacteria can cause more severe or more prolonged illnesses than non-resistant bacteria do (Praff, 1990). Indirect health impacts are less obvious. Mounting evidence suggests that antibiotic-resistant bacteria on food, once ingested, may be able to pass their resistance to other bacteria in the human intestine (Lipsitch *et al.*, 2002). Conjugative plasmids and transposons allow gene exchange between many Gram- positive and Gram-negative bacterial species in the intestine (Teuber, 2001). Danish researchers recently studied volunteers intentionally given chicken or pork contaminated with antibiotic-resistant enterococci; these bacteria persisted in their intestines for at least two weeks. People whose intestines became colonized with drug-resistant enterococci later developed opportunistic infections in hospitals (Lipsitch *et al.*, 2002).



*antibiotic use, selecting resistance

Fig 2. Some routes of transmission for antibiotic resistant gastrointestinal pathogens or normal intestinal flora between animals and humans (adapted from Phillips *et al.*, 2004).

3. MATERIALS AND METHODS

3.1 Collections of samples

3.1.1. Poultry faecal samples

Two hundred eighty cloacal swabs were collected from chicken found in four intensive poultry farms (Farm A, B, C, D) using sterile cotton swabs. The samples were inserted into Amies Transport Medium (Oxoid) and transported to the laboratory using icebox. They were kept at refrigerator temperature until processed.

3.1.2. Cattle faecal samples

Four hundred fifty faecal samples were collected from cattle found in Addis Ababa and some areas in the rift valley (Debre-zeit, Zeway, Shashemene, Wondo-Genet, Awassa). The samples were collected using sterile cotton swabs and sterile stomacher bags. Each sample weighed around 30 gm. The samples were transported to the laboratory using icebox and were processed within 1-2 days after arrival to the laboratory.

3.2 Isolation of enterococci

Faecal samples collected from different poultry farms were streaked on Bile Esculin Agar (BEA) (Ingredients: peptone, 8gm; bile salt, 20gm; ferric citrate, 0.5gm; aesculin, 1gm; agar, 15gm; distilled water, 1000ml; pH, 7.1±0.2). The plates were incubated at 37°C for 24 hours. Enterococci are able to hydrolyze aesculin to aesculetin and dextrose. Aesculetin combines with ferric citrate to form a dark brown or black complex that is indicative of a positive result for enterococci.

3.3. Characterizations of enterococci

Colonies which were considered as presumptive enterococci were further tested for the following characteristics.

3.3.1. Microscopic examination

Microscopic examination was made to study cell shape and arrangement of the isolates. Enterococci are cocci in shape and occur singly, in pairs, or in short chains.

3.3.2. Catalase test

Colonies were flooded with a 3% solution of H₂O₂ (hydrogen peroxide). The formation of bubbles indicated the presence of catalase.

3.3.3. Gram reaction (KOH test)

Test for gram reaction was carried out using KOH test according to Gregerson (1978). Two drops of 3% KOH solution were put to a clean microscopic slide. A colony picked with a clean bacteriological wire loop was stirred in the KOH solution for ten seconds and the inoculating loop was raised slowly from the mass. When KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2 cm or more. Typically, this was observed in Gram-negative bacteria. In cases of no slime, the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria.

3.3.4. Growth in 6.5% NaCl

A colony was picked and transferred with an inoculating loop to test tubes that contained brain heart infusion broth (BHI) which contained 6.5% NaCl. The inoculated broth was then incubated for 24 hours at 35°C. Growth was visually detected.

3.3.5. Growth at 45°C

A colony was picked and transferred to test tubes with BHI and were incubated at 45°C for 24 hours. Growth was visually detected.

3.3.6. Test for carbohydrate fermentation

The test was performed in a broth basal medium containing phenol red as an indicator. Mannitol, sorbitol, raffinose 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 hours. The result was considered positive when the broth turned yellow.

3.4. Isolation and characterization of *Salmonella*

3.4.1. Primary enrichment (pre-enrichment)

On arrival to the laboratory, each cloacal swab was transferred to a tube containing 10 ml of 1% buffered peptone water (BPW) and the swab stick was cut with flamed scissors. These were vortexed and the homogenate was incubated for 18-24 h at 37°C for the metabolic recovery and proliferation of target cells, which could have been injured during processing or to bring the number of target organisms to a detectable level. Faecal samples from cattle (25 gm) were homogenized in 225 ml of buffered peptone water (1%). This was incubated at 37°C for 18-24 hours.

3.4.2. Secondary enrichment

Selenite broth base (SBB) supplemented with 4 gm/l of sodium biselenite (Oxoid) (pH 7.1±0.2), Tetrathionate broth base (TBB) supplemented with 20 ml/l of iodine-potassium iodide solution (pH 8±0.2), Rappaport-Vassiliadis (RVS) (Merck) (pH 5.2±0.2), Maninitol Selenite broth base (MSBB) supplemented with 4 gm/l of sodium biselenite (Oxoid) (pH 7.1±0.2), and Muller Kauffman Tetrathionate broth base (MKTB) supplemented with 19ml of iodine solution and 9.5ml of 0.1% brilliant green solution/l (Oxoid) (pH 8.0±0.2) were used for secondary enrichment. One milliliter of culture from the primarily enriched sample was transferred to test tubes containing 10 ml each of Selenite broth base, Tetrathionate broth base, and Maninitol Selenite broth. Similarly, 0.1ml of the culture was transferred to a tube containing 10 ml each of Rappaport-Vassiliadis and Muller Kauffman Tetrathionate broth. Cultures transferred to SBB and MSBB were incubated at 35°C for 24 hours and those in TBB, RVS and MKTB were incubated at 43°C for 48 hours in water bath.

3.4.3. Solid media

The cultures from each secondary enrichment broth were then streaked on MacConkey Agar No 3 (pH 7.1±0.2), *Salmonella*-*Shigella* (SS) Agar (pH 7.0±0.2), and Xylose Lysine Desoxycholate (XLD) (pH 7.4±0.2) agar plates (all from Oxoid). The plates were then incubated at 37°C for 18-24 hours.

On XLD medium *Salmonella* rapidly ferments xylose and decarboxylases lysine thus altering the pH of the medium to alkaline. Due to the production of H₂S *Salmonella* grows as red colonies with black center. On SSA medium due to the combination of thiosulphate with iron, sulphide production will be indicated by

blackening in the center of the colonies. That is a positive reaction for *Salmonella*. On MacConckey agar No.3 non lactose fermenting colonies produce colorless colonies and *salmonella* does not ferment lactose thus produces colorless colonies

3.4.4. Biochemical identification

Suspected *Salmonella* colonies were picked and purified. Pure cultures were further tested by the following biochemical test.

3.4.4.1. Triple sugar Iron Agar (TSIA) (Oxoid)

The butt was stabbed and the slant was streaked (pH 7.4 ± 0.2) and incubated at 37°C for 18-24 hours, to detect fermentation of sucrose, glucose, lactose and the production of H₂S. For *Salmonella* the butt becomes yellow and gas will or will not be produced and the slant shows a reaction that is alkaline or no change in some cases. H₂S will also be produced.

3.4.4.2. Lysine Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked (pH 6.7 ± 0.2) with a loopful of the culture and incubated at 37°C for 18-24 hours. *Salmonella* produces the enzyme lysine decarboxylase that produces an alkaline reaction (purple color) throughout the medium. Due to the production of H₂S, an intense blackening of the medium will be seen that is a positive reaction for *Salmonella*.

3.4.4.3. Urea Agar (Oxoid)

The slant was heavily streaked (pH 6.8 ± 0.2) and incubated at 37°C for 18-24 hours, to assess the hydrolysis of urea. Urease producing organisms hydrolyze urea to form ammonia and the medium may change to purple red. *Salmonella* does not produce the enzyme urease thus if the color of the urea slant is unchanged that is a positive reaction for *Salmonella*.

3.4.4.4. Simmons Citrate Agar (Oxoid)

The slant was streaked (pH 7.0 ± 0.2) and incubated at 37°C for 18-24 hours, to investigate the utilization of citrate as the sole source of Carbon. If the slant changes to blue color that is a positive reaction for *Salmonella*

3.4.4.5. SIM Medium (Sulphur indole motility test) (Oxoid)

The medium was stabbed (pH 7.3±0.2) and incubated at 37°C for 18-24 hours, to determine H₂S production, motility and production of indole from tryptophan. H₂S production can be seen by blackening of the medium and non motile organisms grow along the line of inoculation where as motile species grow away from the line of inoculation. The production of indole was identified by a red dye complex reaction with Kovac's reagent.

3.4.4.6. Mannitol broth (1%)

Ingredients: Mannitol, 10gm; peptone, 10gm; NaCl, 5gm; phenol red, 0.024gm; distilled water, 1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37°C for 18-24 hours to test fermentation and production of gas.

3.4.4.7. Sucrose broth (1%)

Ingredients: Sucrose, 10gm; peptone, 10gm; NaCl, 5gm; phenol red, 0.024gm; distilled water, 1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37°C for 18-24 hours to test fermentation and production of gas.

3.4.4.8. Glucose broth

Ingredients: Glucose, 10gm; peptone, 10gm; NaCl, 5gm; phenol red, 0.024gm; distilled water, 1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37°C for 18-24 hours to test fermentation and production of gas.

Durham's (fermentation) tubes were added to the carbohydrate tubes to detect the production of gas.

3.5. Isolation of *E. coli* 0157:H7

3.5.1. Sorbitol Mackonkey agar (SMAC)

A loopful of faecal sample was directly streaked on sorbitol mackonkey agar (SMAC) (ingredients: peptone, 20gm; bile salt No3, 1.5gm; sodium chloride, 5gm; neutral red, 0.03ml; crystal violet, 0.001ml; agar, 15gm;

distilled water; pH 7.1 ± 0.2) and incubated at 37°C for 24 hours. Unlike most *E. coli* strains which ferment sorbitol, *E. coli* O157:H7 does not ferment sorbitol thus produces colorless colonies.

3.5.2. Microscopy and KOH test

Colorless colonies were picked and shape of the bacteria was observed using light microscope with an oil immersion objective. Gram reaction was tested by KOH test.

3.5.3. Indole test

Colorless colonies were picked and inoculated in to tryptone water (Oxoid) and incubated at 37°C for 24-48 hours. Kovac's reagent (0.2 to 0.3ml) was then added to this broth culture. The presence of deep red color at the surface of the broth was considered as positive for indole test.

3.5.4. Latex agglutination test

Suspected colonies were further screened by latex agglutination test containing O157 antisera.

3.6. Drug susceptibility testing

3.6.1. Enterococci

Pure cultures were inoculated in to brain heart infusion (BHI) and the cultures were incubated at 32°C for 18-24 hrs. After incubation, the turbidity of the culture was adjusted to 0.5 McFarland Standard to bring the cell density to approximately 10^7 - 10^8 cfu/ml. The McFarland turbidity standard was prepared by mixing 0.1ml BaCl_2 (1%) with 9.9ml H_2SO_4 (1%) (Andrew, 2001).

The isolates were tested for their susceptibility to different antibiotics on Mueller-Hinton agar (pH 7.4 ± 0.2) following the standardized disc diffusion technique with Oxoid drug discs: ampicillin (Amp), (10µg); Sulfamethoxazole/trimethoprim (SXT), (25µg); Penicillin G, (Pen), (10iu); Vancomycin (Van), (30µg); Erythromycin (Ery), (15µg); Doxycycline (Dox), (30µg); Streptomycin (Str), (10µg); Clindamycin (Cli), (2µg); Cephalothin (Cep), (30µg); Amoxycillin (Amx), (25µg); and Amikacin (Ami), (30µg). For the purpose of interpretation, those intermediate cases were considered sensitive.

4. RESULTS

4.1. Prevalence and distribution

4.1.1. Enterococci from poultry

Two hundred eighty samples collected from four different intensive poultry farms (farms A, B, C, and D) were cultured for isolation of enterococci. Four hundred suspected isolates were tested, out of which 234 were found to be enterococci. *Enterococcus faecium* was the dominant species accounting for 49.6% of all isolates followed by *Enterococcus durans* (26.9 %), *Enterococcus hirea* (11.9%) and *Enterococcus faecalis* (11.5%) (Table 1).

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

Farm	No. samples	No. of isolates	Distribution in %			
			<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

4.1.2. Enterococci from cattle

Similarly, from 450 faecal samples collected from cattle, 380 suspected isolates were tested, and 64 were confirmed to be enterococci. Of these, 34 isolates were *E. faecium*, nine were *E. faecalis*, 21 were *E. durans* and none was *E. hirea*.

4.1.3. *Salmonella*

The overall prevalence of *Salmonella* isolates 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. On the other hand, out of 450 cattle faecal samples only 1.8% (8/450) were positive for *Salmonella*

4.1.4. *Escherichia coli* O157:H7

Out of 450 faecal samples, 11 sorbitol negative colonies were isolated from Sorbitol Mackonkey agar (SMAC) plates, but none was confirmed to be *Escherichia coli* O157:H7 by latex agglutination test.

4.2. Antimicrobial resistance

4.2.1. Enterococci from poultry

Of the 234 enterococcal isolates subjected to antimicrobial susceptibility test, using a panel of 11 different antimicrobials, 80.8% showed resistance to penicillin G, 60% clindamycin, 57% cephalothin, 49.6% amoxicillin, or 42.7% to ampicillin. Resistance to the other antimicrobials was less than 40%. All isolates of *E. faecalis* showed susceptibility to amikacin and only a small percentage of isolates from the other three species were resistant to amikacin. All enterococcal isolates showed resistance to all drugs at varying frequencies. Vancomycin resistance was observed in the four species with a frequency between 30 and 54% (Table 2).

Table 2. Percentage of resistance in the four enterococcal species from poultry

Isolates	No. of isolates	Dox	Amx	Pen	Amp	Ami	Van	Ery	Str	Cli	SXT	Cep
<i>E. faecium</i>	116	28.4	44.8	83.6	49.1	7.8	50	37	28.5	63.8	13.8	56
<i>E. faecalis</i>	27	44.4	62.9	85.2	55.6	0	33.3	48.1	37	44.4	18.5	62.9
<i>E. durans</i>	63	25.4	50.8	71.4	44.4	1.6	34.9	34.9	19	49.2	11.1	47.6
<i>E. hirea</i>	28	32.1	53.6	85.7	53.6	3.6	53.6	53.6	46.4	82.1	10.7	78.6
Total	234	29.9	49.6	80.8	42.7	4.74	44	38.7	29.1	59.8	13.3	57.3

Resistance to the different antibiotics tested was observed in the four farms considered in the study. Vancomycin resistance was observed in all the farms with varying frequencies. Half and all isolates from farms A and D showed resistance to vancomycin, respectively. All isolates from farm D also showed resistance to penicillin G, clindamycin and cephalothin (Table 3)

Table 3. Proportion of resistant enterococcal isolates in the four farms

	No. of isolates	Dox	Amx	Pen	Amp	Ami	Van	Ery	Str	Cli	SXT	Cep
Farm A	66	20	40	58	29	6	33	35	22	42	8	34
Farm B	60	18	32	49	32	1	22	25	15	30	5	37
Farm C	67	15	16	36	25	1	8	12	12	20	5	18
Farm D	41	17	28	41	29	3	41	21	19	41	13	41

There was a significant difference among farms in the percentage mean resistant isolates of penicillin G and vancomycin. Farms D, A and B showed the highest percentage mean resistant isolates to penicillin G and farm C showed the lowest. Farms B, A, and D showed significantly higher mean percentage resistant isolates to vancomycin while farm C showed lower mean percentage resistance to vancomycin. The mean percentage of resistant isolates to remaining antibiotics tested did not show a statistically significant difference among farms and species (Table 4).

The present study showed resistance to three or more antibiotics (MDR) in 78.2% of the isolates but the proportion varied with species. The resistance to four and more antibiotics was 64.1%. Resistance to five or more antibiotics was 47%, the percentage of isolates that were resistant to six and more antibiotics were 28.2% and those resistant to 7 and more antibiotics were 15.4%. Those isolates resistant to eight and more antibiotics and nine and more antibiotics were 9.4% and 6.4% respectively. A smaller percentage of isolates were also resistant to $\geq$ ten or eleven antibiotics (Fig. 3).

Enterococcus hirae had the largest proportion of multiple drug resistance (91.2%). *Enterococcus faecalis*, *Enterococcus durans* and *Enterococcus faecium* accounted for 81.5%, 79.4%, and 73.3%, respectively. Fourteen out of 33 enterococcal isolates that showed resistance to three different antibiotics belonged to *E. durans*. Larger proportion of resistance to four, five, six, seven and ten different antibiotics were seen in *E. faecium*. Out of 7 enterococcal isolates that showed resistance to eight different antibiotics, 4 were *E. faecalis*. Only one *E. faecium* isolate showed resistance to all antibiotics tested (Fig 3).

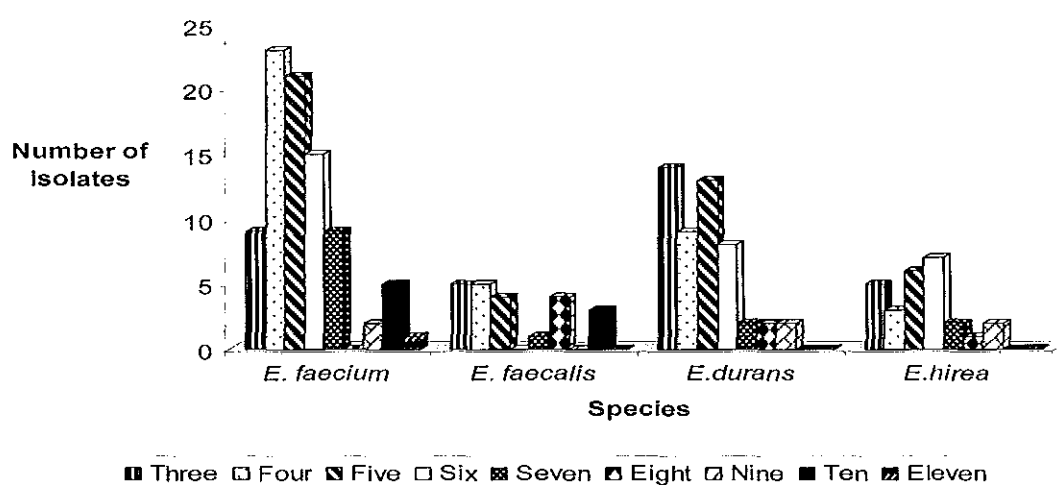


Figure 3. Number of enterococcal isolates from poultry showing multiple drug resistance

Table 4. Percentage of isolates resistant to antibiotics by farm and species with P-values from the analysis of variance

Farms	Species	Dox	Amx	Pen	Amp	Ami	Van	Ery	Str	Cli	Sxt	Cep	Isolates
A	<i>E. faecium</i>	44.12	58.82	88.23	44.12	17.65	52.94	55.88	35.29	70.59	11.76	44.12	34
	<i>E. faecalis</i>	12.5	62.5	87.5	25	0	50	62.5	12.5	50	12.5	50	8
	<i>E. durans</i>	13.33	53.33	80	46.67	0	46.67	33.33	26.67	46.67	13.33	53.33	15
	<i>E. hirea</i>	22.22	77.77	100	55.56	0	44.44	66.67	55.56	77.78	11.11	77.78	9
	All isolates	23.04	63.11	88.9	42.8	4.41	48.5	54.6	32.5	32.5	12.17	53.3	66
B	<i>E. faecium</i>	26.67	46.67	83.33	53.33	6.67	76.67	33.33	30	86.67	30	83.33	30
	<i>E. faecalis</i>	85.71	71.43	85.71	71.43	0	57.14	71.43	85.71	85.71	42.85	85.71	7
	<i>E. durans</i>	6.67	33.33	60	20	0	53.33	33.33	6.67	60	6.67	53.33	15
	<i>E. hirea</i>	25	50	75	62.5	12.5	75	12.5	37.5	87.5	0	75	8
	All isolates	36	50.3	76	52	4.8	65.5	37.6	39.9	79.9	19.8	74.3	60
C	<i>E. faecium</i>	12.12	36.36	75.75	42.42	3.03	36.36	30.3	15.15	42.42	0	48.48	33
	<i>E. faecalis</i>	62.5	50	75	50	0	12.5	37.5	37.5	12.5	12.5	50	8
	<i>E. durans</i>	25	65	70	55	0	25	30	10	40	15	50	20
	<i>E. hirea</i>	66.67	50	66.67	50	0	80	100	83.33	100	16.67	100	6
	All isolates	41.6	50.3	71.8	49.3	0.78	38.5	49.4	36.5	48.7	11	62.1	67
D	<i>E. faecium</i>	31.57	31.58	89.47	63.16	0	26.31	21.05	36.84	52.63	15.79	47.37	19
	<i>E. faecalis</i>	0	75	100	100	0	0	0	0	25	0	75	4
	<i>E. durans</i>	61.54	46.15	76.92	53.85	7.69	15.38	46.15	38.46	53.85	7.69	30.77	13
	<i>E. hirea</i>	20	20	100	40	0	20	40	0	40	20	40	5
	All isolates	28.3	43.2	91.6	64.2	1.9	15.4	26.8	18.8	42.8	10.8	48.3	41
	Farms	p=0.766	p=0.411	p<0.05	p=0.448	p=0.713	p<0.05	p=0.414	p=0.718	p=0.139	p=0.657	p=0.308	
	Species	p=0.895	p=0.311	p=0.260	p=0.638	p=0.361	p=0.474	p=0.619	p=0.672	p=0.248	p=0.885	p=0.267	

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

Among *E. faecium*, 85 isolates showed multiple drug resistance. The resistance was seen in 65 different resistance patterns, the most frequent resistance patterns were seen for four, five and six different antibiotics. Similarly, 22 *E. faecalis* isolates showed MDR and the resistance was seen in 19 different patterns and the most frequent patterns were seen to three and four different antibiotics. Fifty *E. durans* isolates showed MDR and 43 different resistance patterns were seen and the dominant patterns were seen to three and four different antibiotics (Table 5). Among *E. hirea*, 26 isolates showed MDR varying from three to nine different drugs. The resistance was seen in 17 different patterns, and no particular dominant pattern was observed.

Table. 5. MDR pattern in enterococci isolated from poultry

Species	Isolates	MDR Isolates	No. of antibiotics resisted	Dominant resistance pattern		
				Total no. isol.	No. of isolates	Resistance pattern
<i>E. faecium</i>	116	85	Four	23	7	Amo /Pen/Amp/Cep
			Five	21	6	Pen /Amp /Van /Cli /Cep
			Six	15	7	AMO /Pen /Amp /Van /Cli /Cep
			Ten	5	5	Dox /Amo /Pen /Amp /Van / Ery /Str /Cli /SXT/Cep
<i>E. faecalis</i>	27	22	Four	5	3	Amo /Pen /Amp /Cep
			Five	4	2	Amo /Pen /Van /Ery /Cli
<i>E. durans</i>	63	50	Three	14	5	Ery /Str /Cli
			Four	9	3	Amo/Pen /Amp /Cep
<i>E. hirea</i>	28	26				No dominant pattern
Total	234	183				

MDR was observed in the four farms, but it was relatively higher in farm A, 61 out of 66 isolates (92%) and in farm D 38 out of 41 isolates (92%). In farm B, 48 out of 60 isolates (80%) and in farm C, 51 out of 67(76%) isolates showed MDR.

4.2.2. Enterococci from cattle

Of the 64 enterococcal isolates from cattle subjected to nine different antimicrobial agents, more than 70% showed resistance to penicillin G, erythromycin, cindamycin, or amoxicillin. Resistance to vancomycin was observed in all the three species. No isolate was resistant to amikacin.

All *E. faecalis* isolates were resistant to penicillin G and amoxicillin. A higher number were also resistant to erythromycin and ampicillin. The majority of *E. durans* isolates were resistant to clindamycin and cephalothin. Resistance to vancomycin was relatively higher among *E. faecalis* isolates (Table 6)

Table 6. Proportion of enterococcal isolates from cattle resistant to the different antibiotics tested

Isolates	No.of isolates	Resistance distribution in number								
		Pen	Ery	Van	Cli	Str	Ami	Amo	Amp	Cep
<i>E. faecium</i>	34	33	27	5	29	4	0	32	23	9
<i>E. faecalis</i>	9	9	8	4	6	2	0	9	7	3
<i>E. durans</i>	21	20	12	2	19	2	0	19	13	8
All isolates	64	62	47	11	54	8	0	60	43	20

Multiple drug resistance (MDR) was observed in 58 of the 64 enterococcal isolates. All *E. faecalis*, most of *E. faecium* and *E. durans* showed MDR. The highest frequency of multiple drug resistance was observed in *E. faecium* and the most common resistances was to five, seven, four and three drugs in that order (Fig. 4) .

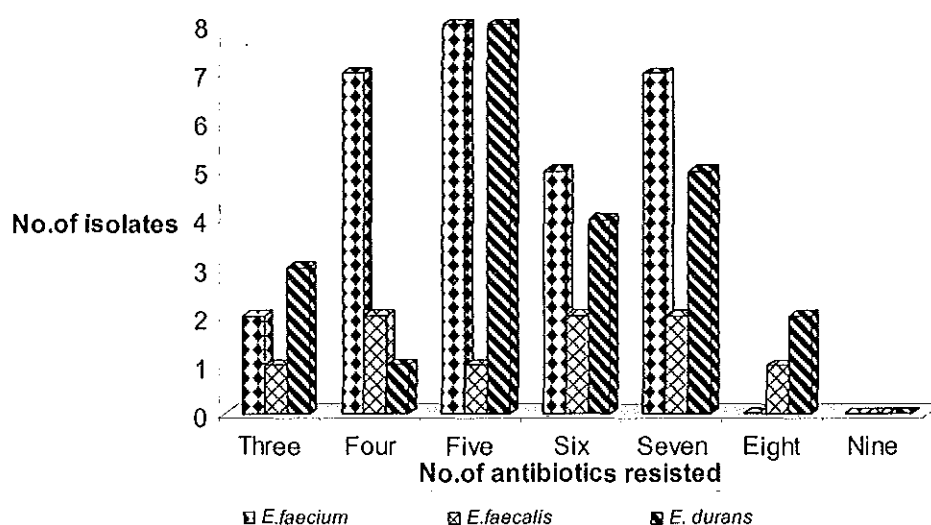


Figure 4. Multiple drug resistance in enterococcal isolates from cattle.

Among *E. faecium*, 29 isolates showed multiple drug resistance. The resistance was seen in 15 different resistance patterns, the most frequent resistance patterns were seen for four, five, six and seven different antibiotics. Similarly, 20 *E. durans* isolates showed MDR, the resistance was seen in 12 different resistance patterns, and the most frequent resistance pattern was seen to five different antibiotics. All the nine *E. faecalis* isolates showed MDR varying from three to eight different antibiotics, and no particularly dominant pattern was observed (Table 7).

Table 7. MDR pattern in enterococci isolated from cattle

Species	isolates	MDR isolates	No. of antibiotics res.	Dominant resistance pattern		
				Total no. isol.	No. of isolates	Resistance pattern
<i>E. faecium</i>	34	29	Four	7	3	Pen /Van /Cli/ Amo
			Five	8	4	Pen/Ery/Van/Cli/Amo
			Six	10	8	Pen /Ery /Van /Cli /Amo /Amp
			Seven	7	5	Pen/Ery/Van/Cli/Amo/Amp/Cep
<i>E. durans</i>	21	20	Five	10	4	Pen/Ery/Van/Cli/Amo
<i>E. faecalis</i>	9	9				No dominant pattern
Total	64	58				

4.2.3. *Salmonella*

Of the 65 isolates of *Salmonella* from poultry tested for an array of eight different antimicrobials, more than 50% (38/56) of the isolates were resistant to streptomycin, none was resistant to amikacin and the resistance to the other antimicrobials was less than 50 % (Fig. 5). Sixteen isolates from poultry showed MDR. The isolates showed resistance to three, four, five and six different antimicrobials. None of the resistance patterns was dominant. (Table 8).

Fig. 5 *Salmonella* isolates from poultry resistant to the antibiotics tested

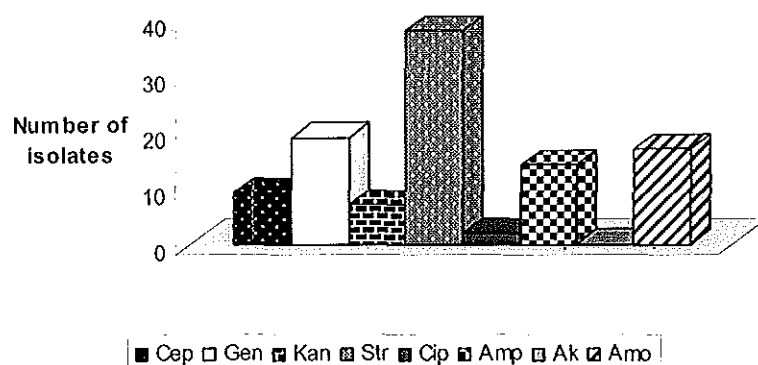
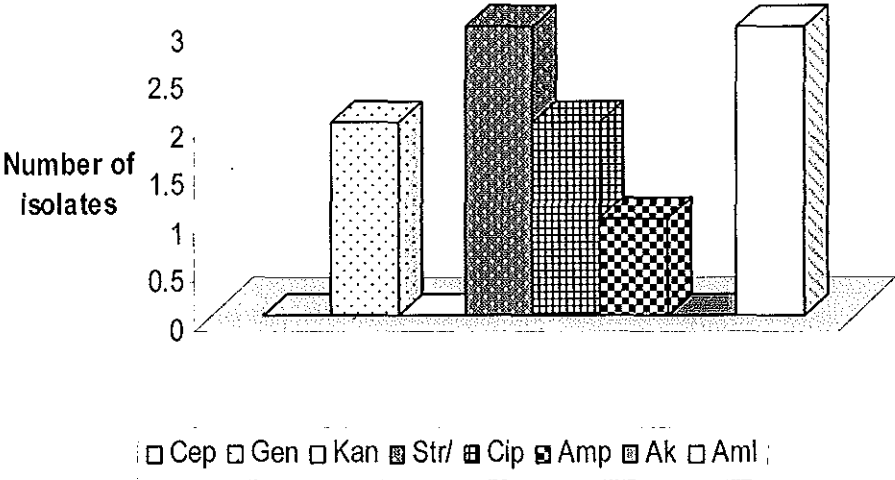


Table 8. MDR pattern in *Salmonella* isolated from poultry

No. antibiotic resisted	No. resistant isolates	The frequent pattern of resistance	
		No. of isolates	Resistance pattern
three	9	2	Kan /Str /Amo
		2	Str /Amp /Amo
		3	Gen /Str /Amo
		1	Cep /Amp /Amo
		1	Cep /Gen /Str
four	4	2	Cep /Str /Amp /Amo
		1	Cep /Gen/ Str /Amp
		1	Kan /Str /Amp /Amo
Five	1	1	Cep /Gen /Str /Amp /Amo
six	2	2	Cep /Gen /Str /Cip /Amp /Amo
Total	16		

From eight *Salmonella* isolates from cattle, three isolates resisted streptomycin and amoxicillin. Resistance to Kanamycin, cephalothin, and amikacin were not observed (Fig 6). Only one isolate showed resistance to Gen /Str /Cip /Amp /Amo.

Fig 6. *Salmonella* isolates from cattle that showed resistance to different antibiotics



5. DISCUSSION

Enterococci form the autochthonous microflora in the intestine of humans and animals, but there is a great diversity in their occurrence. *Enterococcus faecium* is frequently found in the intestinal tract of cattle and fowl. *E. faecalis* is frequently found in fowl and occasionally in cattle. *E. hirea* and *E. durans* occur less frequently in both cattle and fowl (Klein, 2003).

The dominance of *E. faecium* (49.6%) from poultry in our study was a bit higher than that observed in Sweden (35%) (Kuhn *et al.*, 2003). *E. faecalis* was the least dominant in our study (11.5%) but it was the most dominant in Denmark (52%) and Spain (62%) (Kuhn *et al.*, 2003), USA and UK (Hayes *et al.*, 2004). Unlike the studies in Denmark and Sweden in which *E. hirea* stood the second most dominant species (23% and 15%) (Kuhn *et al.*, 2003), *E. hirea* was the third most dominant species in our study (11.9%). The finding of our study showed *E. faecium* was the dominant species, followed by *E. durans*. *E. hirea* and *E. faecalis* covered the remaining share with almost equal proportion. This observation was similar to the study made in Japan (Yoshimura *et al.*, 2004).

Similar studies carried in Sweden and Denmark showed that 50% of the enterococci isolated from cattle were *E. hirea*, followed by *E. faecium*. *E. faecalis* was the least prevalent species in the two countries with 3 and 5% of the isolates, respectively (Kuhn *et al.*, 2003). In our study, however, the dominant enterococcal species in cattle was *E. faecium*, followed by *E. durans*. Smaller proportions of the isolates were *E. faecalis* and none of the isolates were found to be *E. hirea*. This showed that there is no consistency in the dominance of one particular species in different environments.

Salmonella is often present in the intestinal tract of mammals and birds, is readily acquired from feed and environmental sources and contaminates body parts of fowl on the farm. In live chicken, the ceca are the primary predilection site of *Salmonella* colonization. It also often colonizes the cloaca and crop.

Prevalence of *Salmonella* in poultry considered in our study (15%) was higher than that reported from Canada (2-6%) (Chambers *et al.*, 1998) and comparable to the rate in the Netherlands

(13.2%) (Van Pelt *et al.*, 1999), India (15%) (Murugkar *et al.*, 2005), and lower than the rate observed in the USA (20%) (Walinga *et al.*, 2002) and Saudi Arabia (28%) (Barbour and Nabbut, 1982). Isolation of *Salmonella* from these poultry farms is a risk factor for the producers themselves because *Salmonella* can cause economic loss as the infection may result in a high death rate. Those chicken that survive become carriers and contaminate their own eggs (Layers) and meat (broilers) leading to contaminated product supply to the market. The contamination of the egg may lead to egg spoilage and thus lower the hatchability or may remain in the egg to infect newly hatched chick (Barbour and Nabbut, 1982).

The higher prevalence of *Salmonella* in chicken in our study is a serious public health concern because these farms also produced and supplied a variety of products (eggs, whole chicken, sausages, mortadella etc.) to the market in small and poorly equipped slaughtering and food processing plants thus cross contamination of other products by *Salmonella* is highly probable

The prevalence was particularly higher in farm A (13/70) and B (14/70). These farms were bigger farms where around 500 chickens were kept in one block. Presence of the pathogen in few of the chickens would mean that the whole flock in one block would be prone to infection. The free movement of the farm personnel and workers without wearing protective clothes or without changing them when they moved to a new block could contribute to the dissemination of the pathogen within the whole farm.

Farm C and D got day-old chick and laying pullets from farms A and B where the prevalence of the bacterium was higher. Only farm C (11/70) had relatively higher prevalence than farm D (5/70). The practice of using cages (for 4 or 5 chicken) kept one meter above the ground might reduce the cross contamination of uninfected chicken. These could be possible reasons for its reduced prevalence in farm D.

The prevalence of the bacteria was higher in poultry (15%) than in cattle (1.8%), which could be due to keeping a large number of chickens in a small area. Keeping of a large number of chickens in a certain area contributes to the easy horizontal transmission of the pathogen (Barbour and Nabbut, 1982). When infected chicken shed the organism in their faeces, healthy chickens nearby

could ingest it when pecking at contaminated surface of infected birds. This might have contributed to the transmission and higher prevalence of *Salmonella* in poultry than in cattle.

The result in this study shows the prevalence of the *Salmonella* to be 1.8% (8/450). This result is comparable with one study in Ethiopia. Of the 370 faecal samples from slaughterhouse cattle, 1.9% were positive for *Salmonella* (Bayleyegn *et al.*, 2003b). But our observation was lower than the result from dairy herds of USA (27-30%) (Callaway *et al.*, 2005), diarrheic cattle of India (9.6%) (Murugkar *et al.*, 2005) and that of central Texas dairy farms (70%) (USDA, 2005).

Different workers had shown that cattle are the natural reservoirs of *E. coli* O157:H7 (Effler *et al.*, 2001; Bell, 2002; Clallaway *et al.*, 2004). Adult cattle and weaned calves that carry *E. coli* O157:H7 generally remain asymptomatic but shed the bacteria into the environment in their faeces (Gansheroff and O'brien, 2000). The shedding of the bacterium varies with age. Growing cattle 3 to 18 months of age have a higher prevalence of *E. coli* O157 than either younger (suckling) calves or adult cattle (Meerdink, 1999).

Although we did not isolate *E. coli* O157:H7 in this study, studies carried out in different countries had shown the prevalence of *E. coli* O157:H7 in the faeces of cattle. Of healthy cattle faecal samples, 1.5% were positive in Brazil, 3.6% in Denmark and 0.19% in Norway (Johnsen *et al.*, 2001). The prevalence in dairy and beef cattle in the USA was fewer than 10% (Gansheroff and O'brien, 2000). In Washington state, 10 of 3570 (0.28%) faecal samples from dairy cattle were positive for *E. coli* O157:H7 (Hancock *et al.*, 1994). It was also isolated from three (20%) of 15 cattle dung specimens in South Africa (Effler *et al.*, 2001).

E. coli O157:H7 was not encountered in this study. This, however, does not warrant its absence in Ethiopia, where a large number of cattle are present. For instance, there was a report regarding isolation of *E. coli* O157 from cockroaches (2 isolates) collected from some hospitals and restaurants in Addis Ababa (Erdaw Tachbele, 2004). Moreover, *E. coli* O157:H7 was isolated from retail meats in Addis Ababa (1isolate) (Abraham Mikru, 2004). These two findings showed the occurrence of the pathogen in Ethiopia, Thus, large and extensive work has to be done to determine its incidence among the Ethiopian cattle population.

Drugs are given to farm animals for three main reasons: to treat them if they are sick, to prevent disease and to increase productivity. 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 (Wegener *et al.*, 1999). The use of antibiotics for gaining these benefits selects resistant bacteria in the farms.

A study in the United States and Australia on enterococci isolated from poultry at retail market showed that 2% of the isolates were resistant to penicillin, 32% of the isolates showed resistance to erythromycin, 12% were resistant to streptomycin and 70% were resistant to tetracycline (Consumers Union, 2003). From 541 enterococcal isolates recovered from USA, 68% showed resistance to tetracycline, 54 % to erythromycin and 27% to penicillin (Hayes *et al.*, 2004).

In our study, the magnitude of resistance to different antimicrobials was distributed across all *Enterococcus* spp. isolated from poultry except that all *E. faecalis* were susceptible to amikacin. The development of resistance to most antibiotics with different mode of action is considered to be a serious public health concern and the problem becomes more severe in developing countries like Ethiopia.

Resistance of our *E. faecium* isolates to ampicillin (49%) was comparable to isolates from Japan (43%) (Yoshimura *et al.*, 2004), but lower than the isolates from Belgium (94%) (Butaye *et al.*, 2001) and Botswana (97%) (Chingwaru *et al.*, 2003) while it was higher compared to the isolates from USA (1%) (Hayes *et al.*, 2004). The resistances of our isolates to clindamycin (64%) was higher than those from Japan (32.4%) and resistance to erythromycin (37%) was similar (37%) to the Japanese isolates (Yoshimura *et al.*, 2004). Resistance to streptomycin (28%) was lower than the isolates from Japan (90%) (Yoshimura *et al.*, 2004) and Belgium (88%) (Butaye *et al.*, 2001). Similarly the resistance of our isolates to penicillin (84%) was higher than those from USA (79%) (Hayes *et al.*, 2004), and resistance to cephalothin (56%) was higher than those from Botswana (17.3 %) (Chingwaru *et al.*, 2003).

The proportion of our *E. faecalis* isolates resistant to ampicillin (56%) was higher than the isolates from Japan (0%) (Yoshimura *et al.*, 2004) and USA (0%) (Hayes *et al.*, 2004) but lower than those from Botswana (96%) (Chingwaru *et al.*, 2003) and Belgium (100%) (Butaye *et al.*, 2001). The resistance to erythromycin (48%) was lower than those from Japan (76%) (Yoshimura *et al.*, 2004) and USA (69%) (Hayes *et al.*, 2004). The resistance to streptomycin (37%) and clindamycin (44%) was lower than those from Japan (which was 50.2% and 74%), respectively (Yoshimura *et al.*, 2004) and resistance to cephalothin (63%) was higher than those from Botswana (22.4%) (Chingwaru *et al.*, 2003). This species was known to cause most opportunistic infections (Tambyah *et al.*, 2004). In our study, resistance to the ten different antibiotics was observed at varying frequencies but no isolate was found resistant to amikacin. Resistance to most of the antibiotics could result in a serious public health concern.

Vancomycin-resistant enterococci (VRE) are an emerging international threat to public health all over the world. Enterococcal isolates resistant to vancomycin were reported in different parts of the world. Although previous exposure of farms to avopracin selects resistance to vancomycin, the farms considered in this study do not have previous exposure to avopracin (or other glycopeptides like vancomycin) (personal communication).

Vancomycin resistance in our study (44%) was higher than those reported from USA and Australia (13%) (Consumers Union, 2003), New Zealand (5.8%) (Manson *et al.*, 2004), Korea (41.6 %) (Seong *et al.*, 2004) and isolates from organic broilers in Denmark (9.1%) but It was lower than isolates from farms previously exposed to antibiotics in Denmark (74.3%) after the ban of avopracin (Heuer *et al.*, 2003).

The occurrence of vancomycin resistance in 44% of poultry isolates and 17.2% of cattle dung isolates was markedly higher than those isolates from hospitalized patients, out-patients and health care workers from Ethiopia where no vancomycin resistant isolate was found (Amare Worku, 2005). This would indicate that, although vancomycin resistance was not detected in medical isolates, 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. This could eventually undermine the value of this first line drug to treat enterococcal infections in humans.

Although resistance to vancomycin was distributed in all the species isolated in this study, the percentage of *E. faecium* isolates resistant to vancomycin in our study (50%) was higher than the studies from New Zealand (0.8%) (Manson *et al.*, 2004), Botswana (30.7%) (Chingwaru *et al.*, 2003), and USA (0%) (Hayes *et al.*, 2004). Vanomycin resistance of our *E. faecalis* isolates (33.3%) was lower than the result of New Zealand (81.8%) (Butaye *et al.*, 2001) and higher than the results from Botswana (13.1%) (Chingwaru *et al.*, 2003) and USA (0%) (Hayes *et al.*, 2004). *E. durans* (34.9%) isolates from our study showed resistance to vancomycin which was higher than those reported from New Zealand (0.3%) (Butaye *et al.*, 2001). What makes the situation more threatening is the fact that *E. faecalis* and *E. faecium* are the most common causative agents of nosocomial infections and isolates from cattle and poultry had developed resistance to the last drug of choice for infection caused by these species.

The past two decades have witnessed the rapid emergence of MDR enterococci. The resistance of enterococcal isolates in our study to three or more drugs (78.2%), to four or more drugs (64.1%) and five or more drugs (47%) was higher than the isolates from USA and Australia which had values of 38%, 18% and 2%, respectively (Walinga *et al.*, 2002; Consumers Union, 2003.) This might be due to the use of mass treatment during risk of disease outbreak or there might be a practice of adding antibiotics as feed additive to promote the growth of chickens. The resistance genes might be imported from abroad with the live chickens where antibiotic use is part of modern agriculture and dissemination of resistance may be favored by the poor farm management practice in Ethiopia.

Jensen *et al* (2003) showed that identical resistance genes had been found by characterization of the genes in bacteria of food animals and human origin which clearly indicated that the animal and human reservoirs were overlapping and that horizontal transfer of resistance could occur between the two reservoirs.

The proportion of multiple drug resistance isolates in our study was higher than the isolates from hospitalized patients (44%), out patients (23%) and health care workers (19%) from Ethiopia (Amare Worku, 2005). This shows the wide spread dissemination of MDR isolates in food animals (cattle and poultry) and this could be considered as possible risk for acquisition of MDR by human.

Enterococcus faecalis and *E. faecium*, which ranked first and second within the genus as causative agents of nosocomial infections, showed MDR in 73.3% and 81.5% of the poultry isolates, respectively. There is a possibility for this genus to reach the human intestine where proper cooking and hygienic handling of food is not common.

Food animals appear to be important reservoir of the transferable antibiotic resistance (Lukasova and Sustackova, 2003). The ingestion of Glycopeptide Resistant Enterococci (GRE) of animal origin leads to detectable concentrations of the resistant strains in stool of healthy volunteers for up to 14 days after ingestion (Sørensen *et al.*, 2001). Their entry in the human gut allows these strains not only to establish in the human gut but can also favor transfer of their resistance genes to human commensals. Transfer of *van A* and other resistance genes from porcine to human *E. faecium* at higher frequency in the gastric tract of mice had been reported (Moubareck *et al.*, 2003). The transfer has also been reported from enterococci to other gram-positive organisms including streptococci, *Listeria monocytogenes* and *Staphylococcus aureus* (Lukasova and Sustackova, 2003).

Glycopeptides, tecoplanin and avopracin, that are structural analogues of vancomycin and commonly used as animal feed additives, have not been in use both in the human and veterinary settings. Unfortunately, we have 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 the farmers or via Bureau of Agriculture and non-governmental organizations. This poses another concern of environmental dissemination of resistance.

Farm C and D got day-old chicks and pullets from the other two farms. VRE were isolated even from these farms. Vancomycin resistance was seen in all isolates of farm D. Farm D was run by an institution, which had farming not as its sole activity. The poultry farm was an extra activity. The poor management of the farm (for instance, mixing left over feed with fresh feed with the intention of minimizing cost) could contribute to the wide spread dissemination of resistant isolates.

Resistance of enterococcal isolates from food animals to different antimicrobials was reported from different parts of the world. In the present study out of the nine antibiotics tested, resistance to the eight antibiotics was observed in all the three species isolated from cattle. Ampicillin and amoxicillin, in combination with an aminoglycosides, are drugs of choice for treatment of enterococcal infection in human medicine. Unfortunately, higher percentage of isolates was resistant to these drugs. The development of resistance to these drugs especially in *E. faecium* (32/34 for Amo and 23/34 for Amp) and *E. faecalis* (9/9 for Amo and 7/9 for Amp), which are known to cause opportunistic infections, 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.

Salmonella isolates from poultry in different parts of the world showed resistance to different antibiotics. The prevalence of resistant *Salmonella* isolates in our study to Amoxicillin (26.6%) was higher than those isolates from USA (2.9%) (Walinga *et al.*, 2002) but lower than the isolates from India (50%) (Murugkar *et al.*, 2005) and from that previously reported from Ethiopia (45%) (Bayleyegn Molla *et al.*, 2003a). Similarly the resistance of our isolates to Ampicillin (21.5%) was higher than those isolates from USA (2.9%) (Walinga *et al.*, 2002), the Netherlands (9%) (Van Pelt *et al.*, 1999) but lower than the isolates from India (61.8%) (Murugkar *et al.*, 2005) and from that previously done in Ethiopia (45%) (Bayleyegn Molla *et al.*, 2003a).

The resistance of *Salmonella* isolates in our study to ciprofloxacin (3%) was higher than that of USA (0%) (Walinga *et al.*, 2002) and lower than that of India (8.8%) (Murugkar *et al.*, 2005). Similarly, the resistance of our isolates to kanamycin (10.8%) was higher than the study from USA (0%) (Walinga *et al.*, 2002) but lower than that of India (50%) (Murugkar *et al.*, 2005). Meanwhile 29.2% of our isolates showed resistance to gentamycin, which was higher than the results from USA and India (2.9%) (Walinga *et al.*, 2002; Murugkar *et al.*, 2005). The absence of resistance to amikacin in our study was similar to the results previously reported in Ethiopia and that of USA (Walinga *et al.*, 2002; Bayleyegn Molla *et al.*, 2003a).

The finding of almost 25% MDR *Salmonella* isolates in our study was lower than previous study from chicken carcass and giblets in Ethiopia (42%) (Bayleyegn Molla *et al.*, 2003a). The resistance to one or more antibiotics in our study (72.3%) was higher than the studies in USA (5.7%) (Walinga *et al.*, 2002) and Ethiopian isolates from chicken carcass and giblets (63.7%) (Bayleyegn Molla *et al.*, 2003a). Similarly resistances to two or more antibiotics (49.2%), three or more antibiotics (24.6%) and 4 or more antibiotics (10.7%) in our study were higher than those isolate from USA (5.7%) (Walinga *et al.*, 2002). The finding of MDR in chicken from these intensive poultry farms, which are in use for domestic and international market, will pose a serious economic and health problem

Multiple drug resistance in our *Salmonella* isolates from poultry (25%) was almost comparable to those isolates from diarrheal out patients in Ethiopia (22%) (Mogessie Ashenafi and Messele Gedebeu, 1985) but lower than those isolated from clinical specimens from patient in Ethiopia (41%) (Messele Gedebeu and Alebachew Tassew, 1981).

Although our *Salmonella* isolates from cattle were few, their resistance to ampicillin (1/8) was lower than the isolates from diarrheic cattle in India (5/13) but the proportion of isolates resistant to amoxicillin (3/8) was comparable (4/13) (Murugkar *et al.*, 2005). None of our isolates resisted amikacin, which agreed with the results in Texas and New Mexico (USA) (Bischoff *et al.*, 2004; USDA, 2005). None of our isolates resisted cephalthion and kanamycin, which was lower than the isolates from USA (New Mexico) (3%) (Edrington *et al.*, 2004) and that of India (10/13) (Murugkar *et al.*, 2005). The resistance of our isolates to gentamicin (2/8) and ciprofloxacin (2/8) was higher than isolates from diarrheic cattle in India (Murugkar *et al.*, 2005) but the resistance to gentamicin was lower than the result from Japan (4.9%) (Easki *et al.*, 2004).

Ampicillin, cephalthin and kanamycin are commonly used for the treatment of suspected salmonellosis in situations without facilities for culture. Around twenty-one percent of our isolates from poultry showed resistance to Ampicillin which was higher than the isolates from patients with gastrointestinal infections (13%), the percentage resistance of our isolates to cephalthin and kanamycin was comparable with the clinical isolates from Addis Ababa (10%) (Messele Gedebeu and Alebachew Tassew, 1981).

6. CONCLUSION AND RECOMMENDATIONS

Microbiological analysis and safety of food animal with respect to pathogenic microorganisms like *Salmonella* and *E. coli* O157:H7 and commensal microorganisms (enterococci) is of paramount importance due to their role in the food chain of humans.

The food animals considered in this study and their products are used for domestic and international markets as source of income. Beef, milk and poultry products may be consumed raw or improperly cooked. Faecal materials from these animals may be used directly to fertilize agricultural land for production of different vegetables

Monitoring of the microbiological safety of these animals from zoonotic pathogens, the level of resistance of these organisms and the commensal microorganisms to antibiotics used for treatment of infection both in the human and veterinary setting deserves special attention in order to limit the development of resistance and further spread of the resistance genes to the environment. The following specific and important points could be concluded from this study

Enterococcus faecium was the most prevalent species from cattle and chicken, followed by *Enterococcus durans* in chicken and *Enterococcus faecalis* in cattle.

All members of the enterococci isolates showed varying resistance to the antibiotics tested. All *E. faecalis* isolates from chicken and all enterococci from cattle were susceptible to Amikacin.

Resistance to Vancomycin was 44% in enterococcal isolates from chicken and 17.2% in cattle. Resistance to all antibiotics tested was seen in all four intensive poultry farms but the relative percentage of isolates resistant to amikacin was lower in the four farms

Multiple drug resistance was seen in enterococcal isolates from both poultry and cattle with a percentage value of 78.2% and 90.6%. It was seen in all four species from poultry and the relatively least percentage of MDR was seen in *Enterococcus faecium* (73.3%). MDR was seen in 100% of the *Enterococcus faecalis* isolated from cattle (known to cause nosocomial infection). The

percentage of MDR was more than 75% in the four farms but highest in farm A and D (92%). Resistant bacteria in the farms and cattle may not be confined to that specific environment, but can be carried to other environments and would upset the delicate balance of microorganisms in their environment.

Prevalence of *Salmonella* was widespread in different food animals but the magnitude of the problem was high in chicken from intensive poultry farms (15.4%) but the prevalence varied from farm to farm. The problem may likely be complicated by the higher portion of these isolates showing MDR (24.6%). The prevalence in cattle was lower and only one isolate showed MDR. Fortunately *E.coli* O157:H7, a serious food borne pathogen of great concern for the developed world, was not encountered in the present study.

In order to minimize the risk of infection by pathogenic and resistant commensals, consumption of raw or improperly cooked animal food products should be avoided. At the farm level, prudent and judicious use of antimicrobial drugs in veterinary setting has to be encouraged. There should be good farm management practice that includes regular cleaning and disinfection of barns and poultry houses, and replacement or disinfection of used poultry litter. There should be appropriate use of coveralls, boots and head coverings as one move from one block to the other, etc. Farm wastes should not be directly applied to fertilize land. Instead, it has to be applied after composting.

Regulatory agencies should take the issue seriously and address issues regarding importation of live animals, education of farm owners, farm workers in the farm with respect to prudent use of antibiotic, hygiene and safety.

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