Antimicrobial resistance patterns of *Enterobacteriaceae* and *non -Enterobacteriaceae* isolated from poultry intestinal

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Abstract: Gram-negative rods, particularly members of the *Enterobacteriaceae* and non-*Enterobacteriaceae* were the most commonly isolated organisms from poultry intestinal tract in 99 samples. Their prevalence, accurate identification and many biochemical tests had been determined using Analytical profile index (API 20E); (BioMe´rieux, Marcy l'Etoile, France), which were performed with 60 isolates of gram-negative rods, including 7 genera of *Enterobacteriaceae* (10 species). With supplemental testing, API, correctly identified to the genera and species of *Enterobacteriaceae* group as follows: 35 isolates of *Escherishia coli 1* (58.33%), 6 isolates of *Salmonella arizonae* (10%), 6 isolates of *Enterobacter cloacae* (10%), 4 isolates of *Kluyvera sp.* (6.66%), 2 isolates of *Enterobacter aerogenes* (3.33%), 2 isolates of *Klebsiella pneumoniae subsp. pneumoniae* (3.33%), 1 isolate of *Cedecea lapagei* (1.66%), 1 isolate of *Lelercia adecarboxylate* (1.66%) and 1 isolate of *Klebsiella oxycota* (1.66%) and only 2 isolates of non- *Enterobacteriaceae* belonging to *Aeromonas hydrophilla 1* (3.33%). In this study, zone of inhibitions (in mm) of the antibiotics on the test microorganisms were determined and interpreted using standard interpretative chart. *Enterobacter aerogenes* exhibited high resistance to the tested antibiotics with resistance percentage of 94.11% followed by *Klebsiella pneumoniae subsp. pneumoniae* and *Leclercia adecarboxylata* (88.23%), *Enterobacter cloacae* and *Kluyvera sp.* (57.82%), *Aeromonas hydrophilla 1 and Cedecea lapagei* (47.07%). *Escherishia coli 1, Salmonella arizonae* and *Klebsiella oxycota* were the lowest resistant to antibiotics and the resistance percentages were 41.17, 35.29 and 29.41%, respectively. The aim of the study is to determine the prevalence *Enterobacteriaceae* and non *Enterobacteriaceae* species of poultry intestinal tract as well as determine its antimicrobial resistance patterns.
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1. Introduction

Intestinal bacteria play an important role in pathogenesis of intestinal diseases since they are believed to protect against colonization of the intestine by pathogens and to stimulate the immune response of chickens (Mead, 2000).The *Enterobacteriaceae* are a large group of related bacteria living in soil, water and decaying matter, and are also common occupants of both human and animal's large bowel. They are acquired through contaminated food or water, and are the major cause of diarrheal illnesses (Talaro and Talaro, 2002). Poultry feeds can be contaminated directly or indirectly through contact with soil, rodents, birds, dust, human carriers, sewage or water during processing and storage. In microbiological analyses of foods, members of the *Enterobacteriaceae* commonly serve as indicators of faecal contamination and they consist of important zoonotic bacteria such as *Salmonella spp., Yersinia spp.* and *Escherichia coli* (Miranda *et al.,* 2008). Obi and Ozugbo (2007) recorded that *Salmonella* paratyphoid fever in humans have been associated with the consumption of poultry meat from birds that have contracted the infection from contaminated poultry feeds. Aeromonas is a Gram-negative, facultative anaerobic rod that morphologically resembles members of the family *Enterobacteriaceae. Aeromonas spp.* is nowadays classified within the new *Aeromonadaceae* family (Martin-Carnahan and Joseph, 2005). Species of this genus have long been known to cause different type of infection in fish, reptiles and amphibians. Some species, mainly *A. hydrophila*, *A. sobria* and *A. caviae* have been described as emergent foodborne pathogens implicated in human gastroenteritis ranging from mild diarrhea to cholera-like illness (Longa *et al.,* 1996; Vila *et al.,* 2003). Other than gastroenteritis, *Aeromonas* are responsible for meningitis, cellulites, otitis, endocarditis, osteomyelitis, peritonitis, bacteremia, and septicemia, among others diseases (Albert *et al.,* 2000). *Aeromonas* have been reported in untreated and chlorinated drinking water, fresh food, seafood, milk, vegetables, ice cream, and several meats, including pork, beef and poultry (Albert *et al.,* 2000; Abbott, 2003). Bunkova *et al.,* 2010) isolated 88 Gram-negative aerobic and/or facultative anaerobic bacteria from poultry skin, which were subjected to tests: 41 strains of *Enterobacteriaceae* family (11 isolates of *Escherichia coli*, 11 of *Pantoea sp.,* 3 of

Serratia marcescens, 2 of *Serratia liquefaciens,* 1 of *Serratia sp.,* 4 of *Proteus vulgaris,* 1 of *Klebsiella oxytoca,* 3 of *Klebsiella sp.,* 3 of enteric group 69, one strain of *Leclercia adecarboxylata*, one strain of Yersinia enterocolitica), 21 Aeromonas isolates (7 of *Aeromonas caviae,* 2 of *Aeromonas sobria,* 1 of *Aeromonas hydrophila,* 11 of *Aeromonas sp.*), 16 Pseudomonas isolates (eight *Pseudomonas fragi*, four *Pseudomonas putida*, four *Pseudomonas sp.*) and 10 strains of other Gram-negative rods (2 of *Acinetobacter sp.,* 1 of *Delftia acidovorans*, 1 of *Moraxella sp.*, 3 isolates of unspecified Gram-negative aerobic rods, non-fermentative, oxidase-positive and 3 strains of unspecified Gram-negative.

The development of antimicrobial resistance of bacteria has become a global problem. Antibiotic resistance frequencies and profiles seem to vary in different countries and are largely dependent on antibiotic prescribing policies (Gordon, 1980; Adwan *et al.,* 1990). It has been established that several antimicrobial-resistant bacteria obtained from humans have their primary origin as animals raised for human consumption (Aarestrup, 2000) and that these bacteria may contaminate the products derived from these animals (Saenz *et al.,* 2001).

In Saudi Arabia, there have been few documented studies on the contaminating Enterobacteria of poultry feeds and their antibiotics susceptibility patterns. However, the incidence of Enterobacteriosis in poultry farms in Saudi Arabia has been on rise and warrants attention. So the aim of this study is to isolate of Gram negative rods (*Enterobacteriaceae* and non *Enterobacteriaceae*) from intestinal tract of poultry to detect if there is a relationship between poultry feeding which may have been supplemented with antibiotics, and appearance of multi-drug resistant bacteria and its effect on human health.

2. Materials and methods

Sample collection

Ninety nine poultry samples were collected from Faculty of Food and Agricultural Sciences, King Saud University farm and after slaughter their intestinal contents were used for isolation of Gram negative rods (*Enterobacteriaceae and non-Enterobacteriaceae*).

Enterobacteriological analysis

Isolation of Gram negative rods (*Enterobacteriaceae* and no Enterobacteriaceae)

For isolation of Gram negative rod bacteria, MacConkey agar plates media were used. 10 g of the intestinal contents was added to 90 mL saline solution (0.85% NaCl). Dilutions of 10^{-3} and 10^{-5} were prepared and each was poured in triplicate plates. Inoculated MacConkey agar plates were incubated for 24 h at 37 °C. Different appeared colonies were maintained on Nutrient agar slant for further identification by using API 20E.

For isolation of *Salmonella spp.,* 1 g of intestinal content was added to 20 ml lactose broth (Oxoid, CM0137), then the tubes were incubated at 37°C for 24 h, then each 1 mL of the inoculated broth was transferred to tubes containing 20 mL of selenite cystein broth base (Oxoid, CM0699); after addition of sodium biselenite (LP121). The tubes were incubated for 37 °C for 24 h. Selenite cystene inoculated broth was streaked on each of the following media; Salmonella Shigella agar (S.S. agar); Oxoid, CM0099); Xylose lysine deoxycholate agar (XLD agar, Oxoid, (CM0469) Brillinat green agar (Oxoid, CM 329). Plates were incubated at 37 °C for 24 h. The suspected appearing colonies of salmonella were streaked on triple sugar iron agar slant (Oxoid, CM0277), for further identification of API 20E.

Identification of isolates

Each stored isolate was streaked onto Nutrient agar (Oxoid, CM003) plates and incubated overnight at 37°C. One colony was picked and suspended in 5 mL of physiological saline solution (0.85% NaCl) was prepared from each isolate. This material was used to inoculate bioMe´rieux API 20 E strips (bioMe´rieux, Marcy-l'Etoile, France). Strips were inoculated, incubated at 37C for 24 h. and analyzed according to the manufacturer's instructions. Reactions were recorded and identifications were determined by using a computer programme, [API Lab Plus software version 3.2.2 (bioMe´rieux)].

Antimicrobial susceptibility test (AST)

The antimicrobial susceptibility test for each isolate was performed for the total of 10 different genera and species freshly prepared, The tested bacterium was from an overnight culture (inoculated from a single colony) and freshly grown for 4 hours at approximately 10^6 CFU/ml and on dry surfaced Mueller Hinton agar (Oxoid, CM405) using the agar-disk diffusion method (CLSI, formerly NCCLS, 2002). A total of 17 antibiotic discs (Oxoid, U.K) included: Ampicilline 25 µg (AML 25) Chloramphenicol 30 µg (C 30), Kanamycin 30 µg (K30), Doxycycline 30 µg (DO 30), Ciprofloxacin 5 µg (Cip 5), Erythromycin 15 µg (E 15), Sulphamethoxazole trimethoprim 25 µg (SXT 25), Linezolid 30 µg (LZD 30), Vancomycin 30µg (VA 30), Cefadroxil 30 µg (CFR 30), Neomycin 30 µg (N30), Ticarcilline 75 µg (TIC 75), Nalidixic Acid 30 µg (NA 30), Tetracycline 30 µg (TE 30), Colistin sulfate 25 µg (CT 25), Amoxicillin 30 µg (AML 25). Diameters zones of inhibition (mm) were interpreted using the criteria recommended for *Entereobacteriaceae* by NCCLS (2002), Based on the diameter of the antibiotic inhibitory zones, isolates were classified as either sensitive (S) or resistant (R) strains. Interpretation of results was done using the

sizes of inhibition zones. Zones of inhibition ≥ 18 mm were considered sensitive, 13-17 mm intermediate and < 13 mm resistant (NCCLS, 2002; Cheesbrough, 2006; Coyle, 2005; Okonko *et al.,* 2009a, b).

3. Results and discussion

Isolation and identification of *Enterobacteriaceae* and non- *Enterobacteriaceae* from poultry intestinal tract to genera and species level by using API 20 E strips were shown in (Table 1). The most common *Enterobacteriaceae* occurring in the samples were *Escherishia coli 1,* and (35 isolates (58.33%)), 6 isolates of *Salmonella spp.* (10%), 6 isolates of *Enterobacter cloacae* (10%), 4 isolates of *Kluyvera sp*. (6.66%), 2 isolates of *Enterobacter aerogenes* (3.33%), 2 isolteas of *Klebsiella pneumoniae subsp. Pneumonia* (3.33%), 1 isolate of *Cedecea lapagei* (1.66%), 1 isolate of *Leclercia adecarboxylata* (1.66%) and 1 isolate of *Klebsiella oxycota* (1.66%). The non- *Enterobacteriaceae* were 2 isolates of *Aeromonas hydrophilla 1* (3.33%). *E. coli 1* was the predominant organism in intestinal tract of poultry. Edens et al. (1997) reported that two types of putative *E. coli*, called colony type 1 and colony type 2, were isolated consistently from moribund PEMS-afflicted flocks of poults brooded in diverse regions of the State of North Carolina as well as in poults at the North Carolina State. It was of interest that neither colony type 1 nor colony type 2 produced common toxins and that neither colony type expressed any of the known virulence factors tested. Furthermore, these two types of colonies have the capability of penetrating the intestinal epithelial barrier of young turkeys and producing sepsis (Y. M. Saif, Ohio State University, Wooster, OH 44691, personal communication).

Table 1. Percentages of *Enterobacteriaceae* and non-*Enterobacteriacea* from isolated from poultry intestinal tract

Isolates	Nomber of isolates	Percentage %
Escherishia coli 1	35	58.33
Kluyvera sp.	4	6.66
Enterobacter cloacae	6	10
Klebsiella pneumonia spp. pneumoniae	2	3.33
Aeromonas hydrophilla 1	2	3.33
Enterobacter aerogenes	2	3.33
Klebsiella oxycota	1	1.66
Cedecea lapagei	1	1.66
Salmonella arizonae	6	10
Leclercia adecarboxylata	1	1.66

Salmonella sp., was the second abundant *Enterobacteriaceae* found in poultry intestinal tract. This may be due to the normal flora of salmonella in the gasterointestinal tract of poultry or infection caused by a variety of Salmonella species which form one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced production (Talha *et al.,* 2001; Haider *et al.,* 2004;). Avian salmonella infection may occur in poultry either in acute or chronic form by one or more members of Salmonella genus (Hofstad *et al.,* 1992). The motile Salmonellae (paratyphoid group) infection cause Salmonellosis in chickens and have zoonotic significance. Poultry feed may contained high loading of *Enterobacteriaceae* could reach to the poultry directly or during contaminated water. Cox *et al.* (1983), reported that poultry feed (mash and pelleted), meat and bone meal samples were collected from commercial mills and all samples were analyzed for *Enterobacteriaceae* count (ENT) and Salmonella. The genus and species of the various *Enterobacteriaceae* present were also determined. The average ENT for mash, pelleted, and meal samples were log 4.1,.8, and 1.8/g, respectively. *Enterobacteriaceae* were present in 100, 60, and 92% and Salmonella in 58, 0, and 92% of the mash, pelleted, and meal samples, respectively. Overall, the *Enterobacteriaceae* most frequently isolated from all samples were *Enterobacter agglomerans, Enterobacter cloacae*, and *Klebsiella pneumoniae.* Although no Salmonella were found in the pelleted samples, the presence of other *Enterobacteriaceae* suggests that commercial pelleting may not totally destroy Salmonella since their heat resistance is similar to the other organisms found.

Enterobacter cloacae was encountered to be the third type of *Enterobacteriaceae* found in poultry intestinal tract and equally distribution with the ratio of Salmonella (10%). *Enterobacter cloacae* subsp. *cloacae* (*E. cloacae*) occur in the intestinal tracts of humans and animals, in hospital environments, the skin, in water, sewage, soil, meat. *Enterobacter* species include *agglomerans*, *aerogenes*, *amnigenus*, *asburiae*, *cancerogenes*, *cloacae*, *dissolvens*, *gergoviae*, *hormaechei*, *intermedius*, *kobei*, *nimipressuralis*, *pyrinus* and *sakazakii*. This organism may also be isolated from farm animals, primates, fish and insects. *Enterobacter* is frequently isolated from foods like meats, dairy products, poultry and vegetables, since it is common to plants (crops) and animals. Beef and pork products like ground beef, pork sausage, pork loin, and beef steaks are common reservoirs of this organism.

Kluyvera sp. included four isolates (6.66%) isolated from poultry intestinal tract. *Kluyvera* species have also been found in sewage, water, soil samples, a hospital sink, milk and cow (Farmer *et al.,* 1981). Recently, *Kluyvera* species have been isolated from new environmental and animal sources. *Kluyvera* species have been isolated from birds including captive raptors (Bangert *et al.,* 1988). The issue is further clouded by the fact that *Kluyvera* has been recovered

from the gastrointestinal tract of asymptomatic individuals (Fainstein *et al.,* 1981).

2 isolates of *Klebsiella pneumonia* (2.33%) were isolated from poultry intestinal tract. *Klebsiella pneumoniae* has been frequently recovered from birds in which it functioned as a primary pathogen and was associated with respiratory tract disease (Sandra and Duarte, 1998). It can also cause gastrointestinal symptoms. *K. pneumoniae* capsule was found to play no role in colonization of the gut of germ free chickens (Camprubi *et al.,* 1993).

One isolate of *Cedecea lapagei and Leclercia adecarboxylata* (1.66%) also was found in poultry intestinal tract. *Cedecea spp.* has an optimum growth temperature of 37°C but little information about this organism such as what can contribute to its presence is currently available. An unknown *Cedecea sp.* was found on the breast of the organically raised chicken and on the back the commercially raised chicken. *Cedecea lapagei* can be found in different environmental sources associated with poultry processing. Theses bacteria were found on the back of the organically grown chicken (Laura, 2006). *Leclercia adecarboxylata* has been recovered from a variety of foods, water, and animals (snails and slugs) and are most likely ubiquitous in the environment.

Only one isolate of *Klebsiella oxytoca* (1.66%) was found in poultry intestinal tract. *Klebsiella oxytoca* is widely distributed on plants and in the soil, water and is normal flora within the intestines of humans and animals.

The non- *Enterobacteriaceae* 2 isolates of *Aeromonas hydrophila* 1 (3.33%) was found in intestinal tract of poultry. *Aeromonas* have been reported in untreated and chlorinated drinking water, fresh food, seafood, milk, vegetables, ice cream, and several meats, including pork, beef and poultry (Albert *et al.,* 2000; Abbott, 2003). In recent years, *A. hydrophila* has gained public health recognition as an opportunistic pathogen. It has been implicated as a potential agent of gastroenteritis, septicemia, meningitis, and wound infections. It can play a significant role in intestinal disorders in children under five years old, the elderly, and immunosuppressed people. (Handfield *et al.,* 1996; Daskalov, 2006; Health Canada, 2006).

Results in Tables (2, 3) for all bacterial strains showed that *Enterobacter aerogenes* exhibited resistant to 16 tested antibiotics (AMP 25, C 30, K30, DO 30, CIP 5, SXT 25, LZD 30, F 300, VA 30, E 15, N30, TIC 75, NA30, TE 30, CT 30 and AML 25), except CFR 30 which showed an intermediate and the resistance ratio was 94.11%. This ratio was the highest between all strain isolated from poultry intestinal tract. Diene et al. (2012) mentioned that *Enterobacter aerogenes* clinical isolate was resistant to almost all current antibiotics (except gentamicin) commonly used to treat Enterobacterial infections, including colistin. The observed resistance agrees with literature reported that *Enterobacter* species are resistant to most of the antibiotics. The resistance of this species to β-lactam antibiotics, chloranphenicol, quinolones and tetracycline is well documented (Thiolas *et al.,* 2005). There are numerous reports in literature on the increasing resistance of *Enterobacter* species to penicillins and all generations of cephalosporins and their emergence in clinical specimens (Charrel *et al.,* 1996).

Klebsiella pneumoniae subsp. pneumoniae and *Leclercia adecarboxylata* exhibited multiple resistances to most of the tested antibiotics (88.23%). It was found that *Klebsiella pneumoniae subsp. pneumoniae* resistant to 15 kinds of the tested antibiotics (AMP 25, K 30, CIP 5, DO 30, SXT 25, LZD 30, N 30, F 300, VA 30, E 15, TIC 75, NA 30, TE 30, CT 25, and AML 25), intermediate to CFR 30 (15 mm) and only sensitive to C30 (22 mm). These meant that *Klebsiella pneumoniae subsp. pneumoniae* form a multidrug-resistant enteric bacterium which isolated from poultry intestinal tract and these may be due to containing it plasmid mediated multi-resistance genes or the capsules which enveloped the bacteria hampered the action of antibiotics. Kim et al. (2005) were isolated multidrug-resistant enteric bacteria from turkey, cattle, and chicken farms and retail meat products in Oklahoma. Among the isolated species, multidrug-resistant *Klebsiella pneumoniae* was prevalently isolated from most of the collected samples. Therefore, they were isolated of 132 isolates of *K. pneumoniae* and were characterized it to understand their potential roles in the dissemination of antibiotic-resistance genes in the food chains. Multidrug-resistant *K. pneumoniae* was most frequently recovered from a turkey farm and ground turkey products among the tested samples. All isolates were resistant to ampicillin, tetracycline, streptomycin, gentamycin, and kanamycin. Class 1 integrons located in plasmids were identified as a common carrier of the aadA1 gene, encoding resistance to streptomycin and spectinomycin. Production of beta-lactamase in the *K. pneumoniae* isolates played a major role in the resistance to beta-lactam agents. *Enterobacter aerogenes* and *Klebsiella pneumoniae*, two of the most prevalent nosocomial enterobacterial species, can frequently express a multidrug resistance (MDR) phenotype by the acquisition or high-level production of β-lactamases in combination with the overproduction of efflux pumps, associated or not with porin alterations in the outer membrane (Charrel *et al.,* 1996; Malléa *et al.,* 1998; Ardanuy *et al.,* 1998; Gayet *et al.,* 2003; Hasdemir, *et al.,* 2004).

Leclercia adecarboxylata also, appeared to be multi-resistant against 15 kinds of the tested antibiotics (AMP 25, C30, K30, DO 30, CIP 5, SXT 25, LZD 30, F 300, VA 30, E 15, N30, TIC 75, NA30, TE 30, and AML 25) and intermediate to CFR 30 and CT 25 (inhibition zone, was 15 and 13 mm). Sania, (2001) isolated *Escherichia adecaroxylata* (*L. adecarboxylata*, formerly known as *Escherichia adecarboxylata*) twice, once from a camel and once from a chicken. Antibiotic sensitivity was conducted to some *Escherichia spp.* isolated against eight antimicrobial agents. Antibiotic susceptibility was observed namely to cotrimexazole, nalidixic acid, nitrofurantion, ampicillin, tetracycline, gentamycin, colistin sulphate and streptomycin. All the isolates were sensitive to cotrimexazole, nalidixic acid nitrofurantion, gentamycin, streptomycin, tetracycline and colstin sulphate except in sheep (they were resistant to ampicillin). *Escherichia spp.* in camels (*E. coli, E. hermannii* and *E. adecarboxylata*) were highly susceptible to nitrofurantion, nalidixic acid, cotrimexazole, ampicillin and gentamycin, and showed intermediate susceptibility to colistin sulphate, but were resistant to tetracycline and streptomycin. The high incidence of resistance to streptomycin and tetracycline reflects the hazardous of these antibiotics in treatment of camel diseases for long periods in the Sudan.

Enterobacter cloacae exhibited a resistant to 10 antibiotics (K 30, DO 30, CIP 5, E 15, CFR 30, N 30, TIC 75, NA 30, TE 30 and CT 25) and the resistance ratio was (57.82%) for (K 30, CIP 5, E 15, N 30, NA 30 and CT25), intermediate to five antibiotics (SXT 25, LZD 30, F 300, VA 30 and AML 25) and inhibition zones were (15, 21, 15, 14 and 15 mm) respectively, and sensitive to (C 30 and AMP 25) which gave inhibition zones (26 and 20 mm), respectively. Claudia *et al.,* (2012), explained that *E. cloacae* strain isolated from beef carcasses surface showed resistance to four antibiotics from β-lactams group: Ampicillin, Amoxicillin/Clavulanic acid, Cefazolin, Cefoxitin probably by producing β-lactamase. Papadopoulou *et al.,* (1997) showed that the presence of resistant strains of *E. cloacae* to ampicillin, amoxycillin plus clavunalic acid, erythromycin and tetracycline isolated from poultry and hens' egg.

Kluyvera sp. also was exhibited a resistant to 10 of the tested antibiotics (C 30, K 30, DO 30, SXT 25, LZD 30, VA 30, E 15, CFR 30, N 30 and TE 30) and the resistance ratio was (57.82%). It was intermediate to four antibiotics (AML 25, F 300, AMP 25 and CT 25), which gave inhibitions zones (17, 16, 15 and 13mm) respectively, and sensitivity for three antibiotics (CIP 5, TIC 75 and NA 30) and the zones of inhibitions were (22, 21 and 20 mm), respectively.

Aeromonas hydrophilla 1 showed resistant to 8 antibiotics (K 30, CIP 5, F 300, E 15, N 30, NA 30, TE 30 and CT 25) and the resistance ratio was (47.07%). It was intermediate for two antibiotics (CFR 30, DO 30) and the inhibitions zones were (25, 15 mm) respectively. The strain was sensitive to seven tested antibiotics (C 30, LZD 30, AMP 25, TIC 75, VA 30, AML 25 and SXT 25) and gave inhibitions zones (30, 30, 25, 25, 20, 20 and 18 mm) respectively. Our results were agreed with Zanella *et al.,* (2012) who stated that some isolates of *Aeromonas sp.* showed antibiotic resistance to nitrofurantoin, erythromycin, nalidixic acid and tetracycline. This fact must be taken in account considering that these bacteria are potential pathogens, and they can transfer their resistances to other bacteria (Kampfer *et al.,*1999; Rhodes *et al.,* 2000; Vila *et al.,*2002 Song *et al.,* 2004). Antibiotic resistance is particularly relevant in pathogenic *Aeromonas* species in which, besides the classical resistance to-lactamic antibiotics, multiple- resistance has been frequently identified (Kampfer *et al.,* 1999; Vila *et al.,* 2002). Moreover, these bacteria can receive and transfer antibiotic resistance genes, further increasing the risk from resistant bacterial infections (Marchandin *et al.,* 2003).

Cedecea lapagei also, was resistant to 8 antibiotics and equally with *Aeromonas hydrophilla 1* and the resistance ratio was (47.07%) for (K 30, CIP5, E15, CFR30, N30, NA30, TE30 and CT25). It was intermediate to three antibiotics (SXT 25, F 300 and DO 30) and the inhibition zones were (16, 16 and 15 mm) respectively, whereas sensitive to 6 antibiotics (C 30, AMP 25, LZD 30, AML 25, TIC 75 and VA 30) and gave inhibition zone (30, 25, 25, 20, 20 and 18 mm) respectively.

Escherishia coli 1 were resistant to 7 kinds of the tested antibiotics (K30, SXT 25, LZD 30, VA30, E15, CFR30, and CT 25) and the resistance ratio was (41.17%). It was intermediate to three antibiotics (F300, N30 and AML 25) and sensitive to 7 tested antibiotics (C 30, AMP 25, DO 30, CIP 5, TIC 75, NA 30 and TE 30) which gave inhibition zone (30, 20, 20, 20, 20, 20 and 20 mm) respectively. Papadopoulou *et al.,* (1997) isolated antibiotic resistant strain of *E. coli* from poultry to humans to tetracycline, erythromycin, ampicillin and cefalosporins), *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora.

Table 2. Antibiotic sensitivity of 24-h cultures *Enterobacteriaceae* and non- *Enterobacteriaceae* based upon development of inhibitory zone diameters after application of discs containing specific antimicrobial agents

Antibiotics / Microorganisms		AMP 25	C 30	K 30	Do 30	CIP 5	SXT 25	LZD 30	F 300	VA 30	E 15	CFR 30	N 30	TIC 75	NA 30	TE 30	CT 25	AML 25
	R I S	≤13 14-16 ≥17	≤12 13-17 ≥18	≤13 14-17 ≥18	≤10 11-13 ≥14	≤15 16-20 ≥21	≤10 11-15 ≥16	≤20 21-22 ≥23	≤14 15-16 ≥17	≤14 15-16 ≥17	≤13 14-22 ≥23	ND	≤13 14-15 ≥16	≤11 12-14 ≥15	≤13 14-18 ≥19	≤11 12-14 ≥15	ND	≤13 14-17 ≥18
Aeromonas hydrophilla 1		25 S	30 S	0 R	15 I	10 R	18 S	30 S	0 R	20 S	0 R	13 I	0 R	25 S	0 R	0 R	0 R	20 S
Klebsiella pneumoniae spp. pneumoniae		0 R	22 S	0 R	10 R	0 R	0 R	0 R	10 R	0 R	0 R	15 I	8 R	0 R	0 R	0 R	0 R	0 R
Enterobacter cloacae		20 S	26 S	0 R	10 R	0 R	15 I	21 I	15 I	14 I	0 R	10 R	0 R	11 R	0 R	8 R	0 R	15 I
Enterobacter aerogenes		8 R	7 R	7 R	8 R	7 R	7 R	7 R	7 R	7 R	7 R	14 I	8 R	0 R	0 R	0 R	8 R	0 R
Salmonella arizonae		25 S	24 S	15 I	16 I	20 S	20 S	0 R	12 R	0 R	0 R	14 I	14 I	22 S	0 R	20 S	12 R	15 I
Kluyvera sp.		15 I	11 R	12 R	10 R	22 S	10 R	0 R	16 I	0 R	0 R	12 R	0 R	21 S	20 S	0 R	13 I	17 I
Leclercia adecarboxylata		0 R	0 R	0 R	0 R	0 R	9 R	0 R	14 R	0 R	0 R	15 I	10 R	0 R	0 R	0 R	13 I	0 R
Cedecea lapagei		25 S	30 S	0 R	15 I	7 R	16 I	25 S	16 I	18 S	0 R	12 R	0 R	20 S	0 R	12 R	0 R	20 S
Klebsiella oxycota		20 S	25 S	10 R	20 S	25 S	20 S	0 R	15 I	0 R	14 I	13 I	12 R	22 S	20 S	20 S	12 R	14 I
Escherishia coli 1		20 S	30 S	13 R	20 S	20 S	0 R	0 R	16 I	0 R	0 R	10 R	14 I	20 S	20 S	20 S	11 R	14 I

Mean zones of inhibition for common antibiotics tested : ≥ 18mm (S=Sensitive), 13-17mm (I=Intermediate), < 13mm (R=Resistant), except noted above and ND = Not Detected: treated as a common antibiotics inhibition zone. AML 25 =Ampicilline (25 µg)- C 30= Chloramphenicol (30 µg)- K30 = Kanamycin (30 µg) - Do 30 = Doxycycline (30 µg) – Cip 5= Ciprofloxacin (5 µg) E 15 = Erythromycin (15 µg) - SXT 25= Sulphamethoxazole trimethoprim (25 µg)- LZD 30= Linezolid (30 µg)- F300 = Nitrofurantoin (300 µg) - VA 30 = Vancomycin (30 µg) - CFR 30= Cefadroxil (30 µg) - N30= Neomycin (30 µg) - TIC 75= Ticarcline (75 µg) - NA 30 = Naldioxic acid (30 µg) - TE 30= Tetracyclin (30 µg)- CT 25= Colistin sulphate (25 µg)- AML 25= Amoxicilline (25 µg).

Table 3. Percentage of resistant, intermediate and sensitive strains isolated from poultry intestinal tract

Antibiotics resistant strains	Resistant antibiotics	Total resistant (n=17)	% Resistance	% Intermediate sensitivity	% Sensitive
Enterobacter aerogenes	AMP 25, C30, K30, DO 30, CIP 5, SXT 25, LZD 30, F 300, VA 30, E 15, N 30,TIC 75, NA 30, TE 30, CT 30 and AML 25	16	94.11	5.88	0
Klebsiella pneumoniae spp. pneumoniae	AMP 25, K30, CIP 5, DO 30, SXT 25, LZD 30, N 30, F 300, VA 30, E15, TIC 75, NA30, TE 30, CT 25 and AML 25	15	88.23	5.88	5.88
Leclercia adecarboxylata	AMP 25, C 30, K 30, DO 30, CIP 5, SXT 25, LZD 30, F 300, VA 30, E 15,N 30, TIC 75, NA 30, TE 30 and AML 25	15	88.23	11.76	0
Enterobacter cloacae	K 30, DO 30, CIP 5, E 15, CFR 30, N 30, TIC 75, NA 30, TE 30, CT 25	10	57.82	29.41	11.76
Kluyvera sp.	C 30, K 30, DO 30, SXT 25, LZD 30, VA 30, E 15, CFR 30, N 30 and TE 30	10	57.82	23.52	17.64
Aeromonas hydrophilla 1	K 30,CIP 5, F 300, E 15, N30, NA 30, TE 30 and CT 25	8	47.07	11.76	41.17
Cedecea lapagei	K 30, CIP 5, E 15, CFR 30, N 30, NA 30, TE 30 and CT 25	8	47.07	17.64	35.29
Escherishia coli 1	K 30, SXT 25, LZD 30, VA 30, E 15, CFR 30 and CT 25	7	41.17	17.64	41.17
Salmonella arizonae	LZD 30, F 300, VA 30, E 15, NA 30 and CT 25	6	35.29	29.41	32.29
Klebsiella oxycota	K 30,, VA 30, LZD 30, N 30 and CT 25	5	29.41	25	47.05

AMP 25 = Ampicilline (25 µg) - C 30 = Chloramphenicol (30 µg) - K30 = Kanamycin (30 µg) - Do 30 = Doxycycline (30 µg) – Cip 5 = Ciprofloxacin (5 µg) E 15 = Erythromycin (15 µg) - SXT 25= Sulphamethoxazole trimethoprim (25 µg) - LZD 30= Linezolid (30 µg) - F300 = Nitrofurantoin (300 µg)- VA 30 = Vancomycin (30 µg)- CFR 30 = Cefadroxil 930 µg) - N30= Neomycin (30 µg) - TIC 75= Ticarcline (75 µg) - NA 30 = Naldioxic acid (30 µg) - TE 30= Tetracyclin (30 µg) - CT 25= Colistin sulphate (25 µg) - AML 25 = Amoxicilline (25µg)

Salmonella arizonoe was resistant to six antibiotics (LZD 30, F 300, VA 30, E 15, NA 30 and CT 25) and the resistance ratio (35.29%). It was intermediate to five antibiotics (K 30, DO 30, CFR 30, N 30, and AML 25). It was sensitive to 7 tested antibiotics (AMP 25, C 30, TIC 75, CIP 5, SXT 25, TE 30 and AML 25) and gave inhibition zone of (25, 24, 22 20, 20, 20 and 15 mm) respectively. *Salmonella* are among those most known to carry plasmids, which encode for drug resistance. This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobial agents (Molla *et al.,* 2003).

Klebsiella oxycota was resistant to five antibiotics (K 30, VA 30, LZD 30, N 30 and CT 25) and the resistance ratio was (29.41%). It was intermediate to 4 antibiotics (F 300, E 15, CFR 30 and AML 25) and inhibition zones were (15, 14, 13 and 13 mm), respectively. It was sensitive to 8 antibiotics (C 30, CIP 5, TIC 75, AMP 25, DO 30, SXT 25 NA 30 and TE 30)and inhibition zones were (25, 25, 22, 20,20, 20, 20 and 20), respectively. Gundogan, (2011) identified the virulence properties (siderophores, serum resistance, and hemolysin) and antibiotic resistance in extended spectrum β-lactamase (ESBL)-producing Klebsiella isolates from 60 calf and chicken meat samples purchased from various supermarkets in Ankara, Turkey. Of the 45 Klebsiella isolates, 24 (53%) were identified as K. oxytoca and 21 (47%) were identified as *K. pneumoniae*. A high proportion of Klebsiella isolates had virulence factors such as hemolytic activity (67%), siderophore production (44%), and serum resistance (38%). The double-disk synergy test was used to determine ESBL production. ESBL production was detected in 13 (29%) of the 45 Klebsiella isolates. Resistance to 14 antimicrobials was tested in all Klebsiella isolates by the disk diffusion method. All isolates were resistant to two or more antimicrobial agents. All ESBL-producing Klebsiella isolates were highly resistant to cephalosporins and monobactams. Their findings indicate that meat and its products represent potential hazardous sources of multidrug-resistant and virulent Klebsiella species**.**

Conclusion

In summary, higher rates of antimicrobial resistance were observed in *Enterobacteriaceae* and non-*Enterobacteriaceae* strains uisolated from poultry intestinal tract. Due to the more common use of antimicrobial agents in chicken farming consequently higher antimicrobial resistance rates were expected for *Enterobacteriaceae* and non-*Enterobacteriaceae* isolates from poultry feeding on any kind of supplemented feeds (organic or non organic). Therefore the antibiotic selection pressure for resistance in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria. Antimicrobial resistance among the two groups were conform a serious problem in more countries where there is a high frequency of gastroenteric illnesses and many antibiotics fall routinely into inadequate use. Continues survey for antibiotic resistant strains in human, animal and poultry for every country must be taken in account for always detection the new antibiotics which gave high positive results against these bacteria to avoid health risks of using a broad spectrum of antibiotics without any benefit.

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