Antimicrobial resistance profiles of E. coli isolated from pooled samples of Sick, Farm and Market chickens in Nairobi County, Kenya

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Research Article

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Abstract

Background: Antimicrobial resistance (AMR) is an emerging global threat to both human and animal health. This is mainly because the same antimicrobial molecules are used for treatment and prophylaxis in both humans and animals; and about 60% of human pathogens are shared with animals. Thus, a "One health" approach towards combating AMR is critical for the prevention and control of AMR. There is, therefore, need for routine surveillance for AMR in both human and veterinary medicine so as to establish the current status and to formulate strategies on its mitigation. While there are some published data from AMR studies and reviews, data on the prevailing situation, especially in animals, is inadequate. This paper reports on AMR profiles of *E. coli* isolated from chickens in selected parts of Kenya.

Methods: In an effort to establish the antimicrobial resistance profile of bacteria isolated from chicken, A cross-sectional study was carried out in Nairobi County, Kenya. Chickens were purposively selected from three sources: 50 from sick chickens brought to the Poultry clinic at the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for disease diagnosis (post-mortem examination), 50 from a commercial poultry farm in Nairobi, and 72 from a poultry slaughterhouse in Nairobi.

The clinical (sick) cases included broilers, layers, indigenous chickens from various farms, suffering from various disease conditions. Samples from which *E. coli* was isolated, were considered as representative of strains present in other chickens. The commercial farm kept layers under slatted floor (wire mesh) system. Chicken brought to the slaughterhouse were mainly of indigenous type and spent layers,from various parts of Kenya.

Cloacal swabs were taken and the samples placed in Stuart's transport medium and transported to the microbiology laboratory at the department. Isolation of *E. coli* was done by streak-inoculating swab samples on MacConkey agar (Oxoid Ltd) followed by incubation at 37^{o} C overnight.

Results: *E. coli* was recovered from 54/172 samples (31.4%). The study showed that *E. coli* isolates from the chickens were resistant, at varying levels, to some of the tested antimicrobials; particularly to the ones commonly-used, including: Ampicillin (resistance rate of 85.2%), Tetracycline (66.7%), Co-trimoxazole (57.4%) and Streptomycin (40.7%); low resistances were demonstrated with respect to Nalidixic acid at 24.1%; Chloramphenicol at 14.8% (1 isolate). In contrast, high susceptibilities were observed to Amoxicillin and Gentamycin, each at 96.3%. Six (11.1%) of the isolates were resistant to one antimicrobial (Ampicillin) only, and 5 (9.3%) were susceptible to all the 8 antimicrobials tested, while the rest showed variable resistance to multiple antimicrobials, ranging from 2 to 6.

The low *E. coli* recovery of 31.4% (54/172) was contrary to what was expected since *E. coli* lives as a commensal in both humans and animals and is the most commonly-found bacteria (coprobacteria) in the faeces. However, this less-than-100%-recovery using cloacal swab method has been observed in other studies.

This could be due to intermittent shedding of enterobacteria in feces, so if one does not detect the organism with the cloacal-swab method, it does not mean that the animal is completely free of the organism.

Conclusion, this study has demonstrated carriage of antimicrobial resistant *E. coli* in Kenyan chickens; worst-still is the fact that most of them showed multi-resistances ranging from 2 to 6. Evidence of correaltion between antimicrobial usage and development of antibiotic resistant was domenstrated. This information will contribute towards data on current AMR status in bacteria harbored by chickens in Kenya.

Background

Antimicrobials are essential for human health and animal health (www.reactgroup.org), but need to be used cautiously. Livestock (including poultry and fish) health is important for human welfare in two ways: (1) It improves animal welfare, which translates to improved productivity and economic status, thus contribute towards food security and (2) It ensures food safety, since it is estimated that about 60% of bacteria that are pathogenic to humans are from animals/animal products (OIE, 2015), The major problem, with respect to development of antimicrobial resistance, is the fact that same drugs/medicines are used in both humans and animals for treatment or prophylaxis of disease (OIE 2015; GEN 2016, de Souza and Hidalgo 1997; Phillips *et al*., 2004, Gelband *et al*., 2015), and a large percentage of bacteria (pathogenic or not) are shared between the two groups. Prudent use of antimicrobials in animals is, therefore, important as it will control the transfer of antimicrobial resistance between animals and humans (Mitema et al., 2001). Indiscriminate usage of antimicrobials, for example, as growth promoters in veterinary medicine (Hart et al. 2004; Kimera *et al*., 2020; Giguère et al., 2013; Martin et al., 2015) contributes directly to emergence and spread of resistance. Indeed, worldwide, it is estimated that 66% of all antimicrobials are used in farm animals, not people; and that much of this use is routine – used to enable farm animals, mostly pigs and poultry, but sometimes also cattle, to be kept in poor conditions where disease spreads easily (Van Boeckel *et al*., 2015; WHO 2017; Manyi-Loh *et al*., 2018; Yang et al., 2004). In cases of antimicrobial resistance, the resultant food-borne or animal-acquired illness in humans will be less responsive to treatment with respective antimicrobial drug(s).

Since the fight against antimicrobial resistance is of global multitude (Maron 2016; Perovic and Schultz 2016; Teale and Moulin 2012), it is important for each country to establish its current status, and also carry out continuous routine surveillance in order to harness data for action. In Kenya, as in most developing countries, it is difficult to get a complete picture of the AMR situation, especially in animals, as antimicrobial susceptibility testing is not done routinely in diagnostic laboratories (it is only done on specific requests) and specific researches are scarce. It is, however, appreciated that a number of studies on antimicrobial resistance in animals have been carried out in Kenya, and a number of them have been published (Wanja et al., 2020; Mutua *et al*., 2017; Kikuvi *et al*., 2010; Kutto 2012; Gakuya et al., 2007; Njagi *et al*., 2004; Bebora et al., 1994;). There are also reviews by Mitema and Kikuvi (2004) and Kariuki (2011; 2016). The consolidated reports on the situation analysis of AMR in Kenya, by Kariuki (2011; 2016), have covered studies done in humans and animals but require updating, especially with respect to animals. This study has determined extent of antimicrobial resistance in *Escherichia coli*

isolates from pooled samples of three groups of chickens. Chickens were used because they are kept and consumed by many Kenyans and there is also a high tendency of using antimicrobials when the chickens are kept under intensive farming system. *Escherichia coli* was used because it is a common bacterium and also because it is easy to grow and characterize.

Methods

Study design, area and sample chickens

This was a cross-sectional study carried out in Nairobi County, Kenya. It comprised chickens purposively selected from three sources: veterinary poultry clinic, poultry commercial farm and poultry market slaughterhouse. A total of 50 sick chicken that were brought to the Poultry clinic at the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for disease diagnosis (post-mortem examination; regardless of their disease condition), 50 from a commercial farm in Nairobi, and 72 from a slaughterhouse in Nairobi.

The clinical cases included broilers, layers, indigenous chickens from various farms, suffering from various disease conditions (not necessarily caused by *E. coli*) including: septicaemia, pneumonia, coccidiosis, New castle disease, Gumboro disease, fowl pox, leucosis, nutritional deficiency, aflatoxicosis, yolk-sac infection, helminthosis, ectoparasites, trauma (e.g. liver rupture). Like healthy chickens, the clinical cases carry *E. coli* in their guts, as commensals; the isolated *E. coli* were, therefore, taken as representatives of strains present in other chickens in the respective farms. Market birds were mainly of indigenous type and spent layers, brought to the slaughterhouse from various parts of Kenya. The farmed chickens were from one farm which was keeping layers under slatted floor (wire mesh) system.

Sample collection, handling and transport

Cloacal swabs were taken from 50 chickens brought to the clinic during the study period, 50 from commercial poultry farm and 72 market (slaughterhouse) The samples were then placed in Stuart's transport medium and transported to the microbiology laboratory at the department of Veterinary Pathology, Microbiology and Parasitology for bacterial isolation and identification.

Isolation and identification of *E. coli*:

Isolation of *E. coli* was done by streak-inoculating swab samples on MacConkey agar (Oxoid Ltd) followed by incubation at 37^{o} C overnight. Organisms from lactose-fermenting (pink) colonies were phenotyped and confirmed as *E. coli* through Gram-staining, growth on Eosin Methylene Blue agar, and testing for motility and biochemical reactions, including: Indole, Methyl red, Voges Proskauer, Citrate, Urease; interpretation done using the criteria given in Bergey's Manual of systemic bacteriology (Holt *et al*., 1994).

Antimicrobial susceptibility testing of the *E. coli* isolates;

Antimicrobial susceptibility testing was done by Agar Disk Diffusion method as previously described by Bauer *et al*. (1966) and recommended by the Clinical and Laboratory Standards Institute (CLSI; 2020).

The *E. coli* isolates were tested for susceptibility against 8 antimicrobials, commonly-used for treating bacterial infections in both humans and animals; including: Ampicillin (AMP; 25 ug); Tetracycline (TE; 25 ug), Co-trimoxazole (COT; 25 ug), Streptomycin (S; 10 ug), Nalidixic acid (NA; 30ug), Amoxycillin (AMC; 30 ug), Gentamycin (GEN; 10 ug), Chloramphenicol (C; 30 ug) (Oxoid, Basingstoke, United Kingdom). After incubation at 37º C overnight, the diameters of the growth-inhibition zones around the discs were measured. *E. coli* - ATCC 25922 (WHO, 2003**),** was used as the reference strain. Guidelines provided by the CLSI (2020).

Results

Escherichia coli isolated from chickens

Escherichia coli organisms were isolated from a total of 54 chickens [prevalence of 31.4% (54/172): 36 from the 50 clinical cases sampled (prevalence of 72%); 11 from the 50 farm chickens sampled (prevalence of 22%) and 7 from the 72 market/slaughtered chickens sampled (prevalence of 9.7%)].

Antimicrobial susceptibility/resistance test results for the 54 E. coli isolates

The organisms showed highest resistance to Ampicillin at 85.2%, followed by Tetracycline at 66.7%; Co-trimoxazole at 57.4%; Streptomycin at 40.7%. Low resistances were demonstrated to Nalidixic acid at 24.1%; Chloramphenicol at 14.8% (1 isolate); while high susceptibilities were observed for Amoxycillin and Gentamicin, each at 96.3%. There were 6 (11.1%) isolates that were resistant to one antimicrobial (Ampicillin) only, and 5 (9.3%) that were susceptible to all the 8 antimicrobials tested, while the rest showed variable resistances ranging from 2 to 6 antimicrobials. Antimicrobial susceptibility test results of the 54 *E. coli* isolates are shown in Table 1. Figure 1 gives graphical representation of antimicrobial resistance rates for the test isolates.

Table 1
Antimicrobial susceptibility/resistance patterns of the isolated E. coli: n = 54

Antimicrobial	Susceptible		Resistant	
	Number	Percent	Number	Percent
Ampicillin (Amp)	8	14.8	46	85.2
Tetracycline (TE)	18	33.3	36	66.7
Co-trimoxazole (COT)	23	42.6	31	57.4
Streptomycin (S)	32	59.3	22	40.7
Amoxycillin (AMC)	52	96.3	2	3.7
Gentamycin (GEN)	52	96.3	2	3.7
Chloramphenical (C)	46	85.2	8	14.8
Nalidixic acid (NA)	41	75.9	13	24.1

Multidrug resistance in E. coli **isolates**:

Forty-four out of the 54 (81.5%) *E. coli* isolates showed multidrug resistance (resistant to two or more antimicrobials). Figure 2 presents number of organisms resistant to respective number of antimicrobials; antimicrobial combinations resistant-to are given in Table 2; while Fig. 3 shows the number of times an antimicrobial was involved in cases of multi-drug resistance among the *E. coli* isolates. Ten (22.7%) of the multi-drug-resistant isolates were resistant to 2 antimicrobials; 15 (34.1%) were resistant to 3 antimicrobials; 8 (18.2%) to 4, while 5 (11.4%) each were resistant to 5 and 6 antimicrobials, respectively (Fig. 2). Of the 152 times that the test antimicrobials were included in multi-drug combinations, the antimicrobial included most was Ampicillin at 26.3% (40/151); followed by Tetracycline at 23.7% (36/152); Co-trimoxazole at 20.3% (31/152); Streptomycin at 14.5% (22/152); Nalidixic acid at 7.9% (12/152); Chloramphenical at 5.3% (8/152); Gentamicin at 1.3% (2/152) and lastly Amoxicillin at 0.7% (1/152) (Fig. 3).

Table 2
Multi-drug resistant patterns demonstrated by the test isolates

Number of antimicrobials resistant-to	Number of isolates resistant to respective number of antimicrobials	Resistant antimicrobial combinations
2	10	One had combination of COT–TE Six had combination of TE-AMP One had combination of S-TE One had combination of COT-AMP One had combination of S-AMP
3	15	Four had combination of COT-S-AMP Eight had combination of COT-TE-AMP One had combination of NA-S-TE One had combination of C-TE-AMP One had combination of C-S-AMP
4	8	Five had combination of COT–S-TE-AMP Two had combination of COT-NA-TE-AMP One had combination of NA-S-TE-AMP
5	5	One had combination of COT–NA-GEN-TE-AMP Two had combination of COT-NA-S-TE-AMP Two had combination of COT-C-S-TE-AMP
6	5	Three had combination of COT–C-NA-S-TE-AMP One had combination of COT-C-NA-GEN-TE-AMP One had combination of COT-NA-S-AMC-S-TE-AMP

COT – Co-trimoxazole; TE – Tetracycline; AMP – Ampicillin; S – Streptomycin; NA – Nalidixic acid; C –Chloramphenicol; GEN – Gentamycin; AMC – Amoxicillin

Discussion

This study was carried out to demonstrate the current antimicrobial resistance level in *E. coli* organisms isolated from chicken cloacae, from selected study sites in Nairobi. There was a low *E. coli* recovery of 31.4% (54/172). This was contrary to what was expected since *E. coli* lives as a commensal in both humans and animals; it is the most commonly-found bacteria (coprobacteria) in the faeces (Buxton and Frazer, 1977). However, this less-than-100%-recovery using cloacal swab method has been observed in other studies. Ibrahim et al., (2019) isolated *E. coli* at 53.4% (269/504); Bebora (1979) isolated the organism from 4 lots of chickens at 51.1% (97/133); 46% (98/176), 66% (66/100) and 88% (22/25). There is documentation on intermittent shedding of enterobacteria in feces has previously been documented (Magwood and Bigland 1962; Brownell *et al*., 1969; Smith *et al*., 1972; Brown *et al*., 1975); Shedding is influenced by stress: muscular fatigue, cold, wetness, limitation of food and water, concurrent infection (Brownell *et al*., 1969). Working on *Salmonella* Typhimurium, Brownell *et al*., (1969) found that cloacal excretion of the organisms occurred during the first 5 days of infection, after which the excretion dropped considerably. Williams and Whittemore (1976) had similar findings; they also concluded that cloacal swab method was inadequate for isolation of *Salmonella* Typhimurium. The amount of fecal material in the cloacal swab is much less than in intestinal swab, so there is higher chance of not picking the organism, even though present.

Results of this study showed that *E. coli* isolates from the screened chickens were resistant, though at varying levels, to some of the commonly-used (because they are cheap, hence affordable to the inhabitants of the study area). They included: Ampicillin (resistance rate of 85.2%), Tetracycline (66.7%), Co-trimoxazole (57.4%) and Streptomycin (40.7%) (Table 2). The resistance may have developed as a result of high or indiscriminate usage of antimicrobials in the area; either by the humans or on their animals; it may also be as a result of environmental contamination through human/animal trafficking across the area (through fecal contamination, spitting or other excrements) or through careless disposal of medicines. This trend of resistance has also been reported in other studies (Bebora, 1987; Ombui et al., 2000; Mapeney *et al*., 2006; Gakuya et al., 2007, Kikuvi *et al*., 2007b; Allorechtova *et al*., 2012). In this study, it was encouraging to find that there were some bacterial strains that were still susceptible to the commonly-used antimicrobials, for example: 5 (9.3%) of the isolates were susceptible to all the 8 antimicrobials tested. High susceptibilities were observed to Amoxycillin and Gentamycin (each at 96.3%), Chloramphenicol (85.2%) and Nalidixic acid (75.9%).

The presence of zoonotic antimicrobial resistant bacteria in dogs (Guardabassi 2004; Allorechtova *et al*., 2012) and rats (Gakuya et al., 2007); animals that occur widely in human and chicken environment (Stregowski 2017; Cleaveland et al 2006; Blackburn *et al*., 2014; Lembo *et al*., 2011; Wareth *et al*., 2016) - they are everywhere – in human dwellings (especially in informal settlements), in markets, in farms; so, they can easily acquire and disseminate antimicrobial resistant bacteria. Allorechtova *et al*., 2012 specifically looked-for ESBL-producing *E. coli* strains in Northern Kenya and demonstrated their presence in humans, dogs and, to a lower extent, cats. Comparing genetic profiles of the ESBL-producing *E. coli* isolates, 8 isolates from dogs and 2 isolates from humans gave identical profiles; while a close relationship (> 95% relationship) was found in one human isolate and one cat isolate. This demonstrates spread of resistant bacteria between humans and dogs; some of them were found to be multi-resistant.

Many classes of antimicrobials have been used to treat both humans and livestock (Phillips *et al*., 2004). They include: β-lactams (Penicillins and Cephalosporins); Sulphonamides with or without Trimethoprim; Tetracyclines; Macrolides, Lincosamides and Streptogramins; and Quinolones including Fluoroquinolones (Bager and Emborg, 2001). Classes most used to treat livestock are: Penicillin derivatives, such as Ampicillin and Cloxacillin; Sulphonamide, e.g. Tylosin, used for treatment of metritis and acute mastitis in cattle, sheep and goats, enteritis, pneumonia, erysipelas, and infectious arthritis in swine (Giguère et al., 2013). Tylosin is also used to treat chronic respiratory disease in chickens. Tetracycline and Co-trimoxazole (containing sulfamethoxazole and trimethoprim) are two most-used antimicrobials for prophylaxis and as growth promoters in livestock rearing, so as to increase productivity (Giguère et al., 2013).

In Kenya the most commonly used medicine for treatment of proutry is about 45 different types with over 62% identified as antimicrobials, which includes Fosbac® (fosfomycin and tylosin), Tylodox® (Tylosin tartrate 100 mg and Doxycycline hyclate 200 mg), Limoxil® (Oxytetracycline), Tylodoxine® (Doxycycline and Tylosin Tartrate), ampicillin, tetracycline, sulphamethoxazole and co-trimoxazole and Tylosine 75® (Tylosin tartrate Eq. 750 000 I.U. and 750 mg Tylosin). The additional remained basic multivitamins, probiotics, and dewormers (Kiambi *et al*., 2020; Afakye *et al*., 2021). Therefore, detection of bacteria that are resistant to these antimicrobials is not surprising; meaning that treatments with such antimicrobials are unlikely to be effective. Resistance, particularly to the commonly available antimicrobials, poses a major health concern, as alternative therapeutic choices are either unavailable or too expensive to be affordable for most patients (Kariuki *et al*., 2010).

Increased use of antimicrobials mainly for prophylaxis and as growth promoters in animals in Kenya is encouraged by the increased demand in milk, meat, eggs, due to increased population and popularization of the products (Manyi-Loh *et al*., 2018; Van den Bogaard *et al*., 2001).

Most of the antimicrobials are used in intensively-kept chickens and pigs, while in other livestock, more antimicrobials are used in treatment and prevention of mastitis. The Ministry of Agriculture, Livestock, Fisheries and Irrigation animal census (2017) gives chicken population to be at 48,123,577 (broilers 3,819,515; layers 4,237,188; indigenous 40,067,874). The estaimated increases and the high consumption of antimicrobial use in chicken, is correspondingly with reference to given evidence of non- prudent use of antimicrobials in chicken in Kenya. Antimicrobials are commonly purchased deprived of instructions, exposing incorrect practice or purschasing the wrong drug which is self-administered by un-professional i.e. farmers (Kiambi et al., 2021).

The situation is made worse since human doctors and veterinarians tend to use antimicrobials to cover themselves in case of wrong diagnosis or as a cover for any secondary bacterial infection; they use the assurance that: “if it is broad-spectrum, it can shoot better” (Chhorvoin et al., 2016). This, coupled with increased use of antimicrobials in humans, mainly to treat respiratory, enteric and hospital acquired infections (Bururia 2005; Kariuki et al., 2006;2007; Oundo et al., 2008), and ease of acquiring the antimicrobials over the counter (i.e. purchase without prescriptions) (Ayukekbong *et al*., 2017) has

contributed to the sky-rocketing levels of antimicrobial resistance experienced today. Most farmers practice mixed animal-raising; that is: they keep many types of animals; there is also close relationship between humans/farmers and their animals; so, resistant bacteria can easily be transferred across the animals and to/from humans.

Eighty-one point five percent (81.5%; 44/54) of the *E. coli* isolates, in this study, showed multidrug resistance; Ten (22.7%) of the multi-drug-resistant isolates were resistant to 2 antimicrobials; 15 (34.1%) were resistant to 3 antimicrobials; 8 (18.2%) to 4, while 5 (11.4%) each were resistant to 5 and 6 antimicrobials, respectively (Fig. 2). Of the 152 times that the test antimicrobials were included in multi-drug combinations, the antimicrobial included most was Ampicillin at 26.3% (40/151); followed by Tetracycline at 23.7% (36/152); Co-trimoxazole at 20.3% (31/152); Streptomycin at 14.5% (22/152); Nalidixic acid at 7.9% (12/152); Chloramphenical at 5.3% (8/152); Gentamycin at 1.3% (2/152) and lastly Amoxycillin at 0.7% (1/152) (Fig. 3). This further demonstrates the resistance pattern as being towards the cheap-commonly-used antimicrobials; echoing the worldwide worry towards antimicrobial resistance (GEN 2016; Maron 2016; Perovic and Schultz 2016). Multi-drug resistance has been reported by a number of researchers in Kenya; in animals – Bebora (1987), Ombui et al. (2000), Mapeney *et al*. (2006), Gakuya et al. (2007), Kikuvi *et al*. (2007b), Allorechtova *et al*. (2012), Igizeneza *et al*. (2020), Wanja et al. (2020); in environment – Wambugu *et al*. (2015), Kutto (2012); in humans – Kariuki *et al*. (1996; 2006), Bururia (2005), Oundo et al. (2008). It has also been reported by many researchers outside Kenya (Van den Bogaard *et al*. 2001; Ryu *et al*. 2012; Adzikey *et al*. 2012; Nys et al., 2004; Kennedy and Collington (2010); Ulstad *et al*., 2016; GEN 2016).

Conclusion

This study has demonstrated carriage of antimicrobial resistant *E. coli* in Kenyan chickens; worst-still is the fact that most of them showed multi-resistances ranging from 2 to 6 antimicrobials the number could have been even higher if more antimicrobials were tested. Data from this study will thus contribute towards buidling on current AMR profiles in bacteria harboured by chickens/animals in Kenya and helpinform policymakers in their fight against AMR.

Declarations

Data statistic anlysis

The statistical anlysis was done using R statisrical program. Descriptive satistics appropriate hypothesis tests were carried out to establish the association and correlations between antimicrobial resistance and the selected variables.

Availability of data and materials

The data used and analysed in this study are obtainable from the corresponding author on rational demand.

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Contributions

TAD, LCB, MOO, SK, GMM and PKG and Conceptulalized and study design. TAD, LCB, MOO and SK planned and completed data collection.GMM did the statistical analysis. LCB, TAD and MOO wrote the first draft of the manuscript. TAD, LCB, MOO, GMM, SK and PKG Wrote, reviewed and edited the manuscript. LCB, MOO, PKG, SK and GMM contributed technically and financially towards the completion of the study. All authors have read and agreed to published the manuscript.

Ethics approval

This research was conducted after obtaining ethical approval of research proposal by Biosafety, Animal Use and Ethics Committee (REF: FVM BAUEC/2016/104), Faculty of Veterinary Medicine, University of Nairobi, Kenya. This research was prepared in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments in Animals.

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Consent for publication

All authors declare consent for publication the research

Conflicts of Interest:

The authors declare no conflict of interest.

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Figures

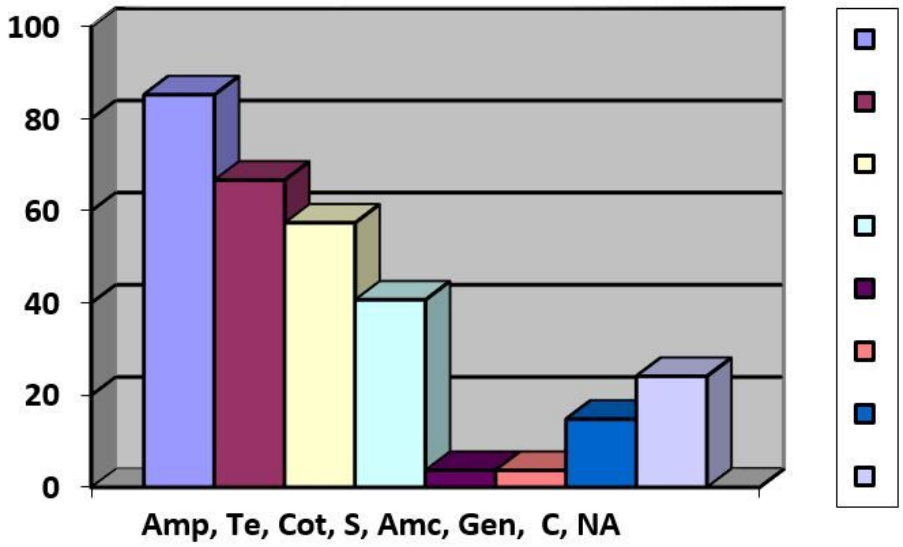

Figure 1

Graph showing resistance rates (%) per antimicrobial. From left to right: Ampicillin, Tretracycline, Co-trimoxazole, Sreptomycin, Amoxycillin, Gentamycin, Chloramphenicol, Nalidixic acid, respectively

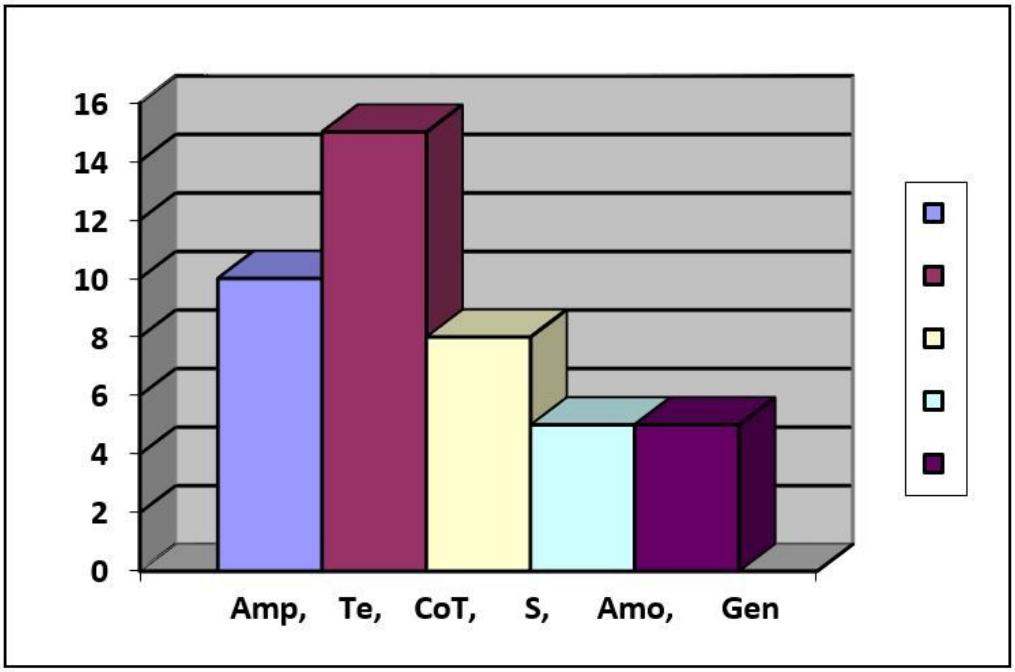

Figure 2

Number of *E. coli* isolates multi-resistant to respective number of antimicrobials From left to right: 2, 3, 4, 5, 6 antimicrobials

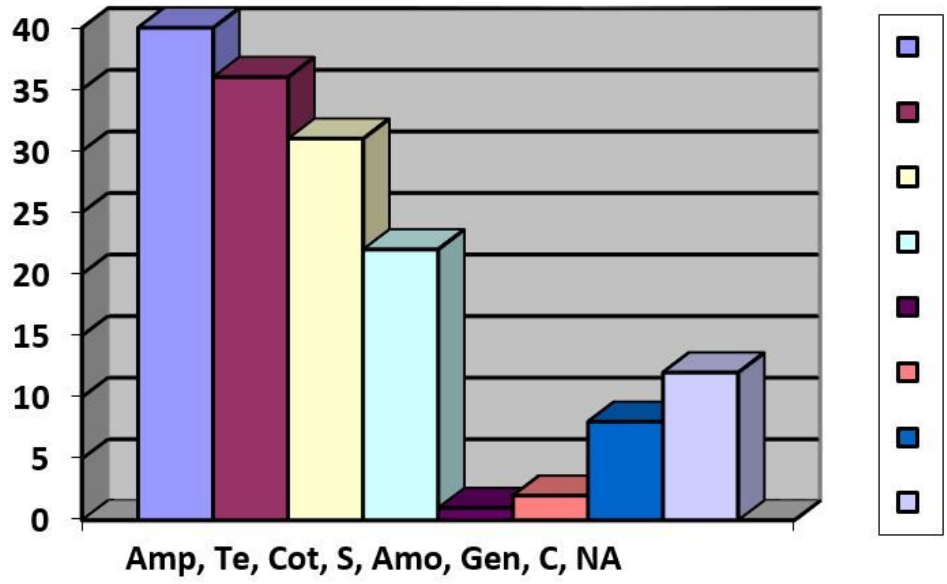

Figure 3

Number of times an antimicrobial was involved in cases of multi-drug resistance among the *E. coli* isolates. From left to right: Ampicillin, Tretracycline, Co-trimoxazole, Sreptomycin, Amoxycillin, Gentamycin, Chloramphenicol, Nalidixic acid, respectively