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A study on the antimicrobial resistance profile of *E. coli* isolated from dairy farm sewage

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Abstract
The increase in demand for livestock products and the development of farming, nutrition and management have led to changes in the animal production systems. The intensification of food production systems rules out tolerance of disease outbreaks; thus, various antimicrobial drugs are administered for the prevention of diseases. Antimicrobials are administered in the control treatment called metaphylaxis in order to avoid the spread of disease. Due to the spatially limited environment and repetitive production scheme, large breeding farms form closed enclaves, where the pool of the resistant microorganisms and resistance genes can be accumulated and circulate between animals and their environment. It is interesting to what extent the supply of antimicrobials results in the development of resistance and whether after the cessation of antimicrobial pressure resistance decreases. Keeping inview of the development of antimicrobial resistant *E. coli* the present investigation was carried out to detect the resistance patterns of *E. coli* isolated from dairy farm sewage. A total of 128 sewage samples were collected from organized, unorganized dairy farms and also from animal sheds of small farms and isolated *E. coli*. All the 128 isolates were confirmed as *Escherichia coli* by subjecting them to various biochemical tests. Antibiogram pattern was studied for the isolates using 14 antibiotic discs, which revealed varying degree of resistance to ampicillin (92.19%), Penicillin-G (76.56%), cefotaxime (71.10%), streptomycin (64.84%), gentamicin (64.06%), chloramphenicol (57.81%), tetracycline (54.69%), cefoperazone (51.56%), ofloxacin (44.53%), cefadroxil (39.06%), ciprofloxacin (38.28%), azithromycin (30.40%), meropenem (27.34%) and tigecycline (20.31%).

Keywords: *E. coli*, Dairy farm sewage, Antimicrobial Resistance Profile

1. Introduction
Escherichia coli belongs to the family Enterobacteriaceae, is a gram negative, motile, non-sporulating, robust, facultative anaerobic and rod shaped bacteria [74]. These are naturally colonizes in the digestive tract of animals and humans as a normal microflora [17]. Although most of the *E. coli* isolates are harmless, which are inhabitating the intestines of humans and animals [32], whereas 10 to 15% of coliforms are opportunistic and pathogenic serotypes [20]. Cattles and sheep are the major reservoir for *E. coli* [17, 64]. Pathogenic *E. coli* especially *E. coli* O157:H7 is responsible for the occurrence of severe outbreaks since the past two decades [11]. Shiga toxin producing *E. coli* related disease may involved in either sporadic cases or large outbreaks involving a common contaminated food source. In some cases individuals infected with STEC may be asymptomatic, even though large numbers of organisms and free toxins were found in the faecal samples of the individuals [19, 25]. Most of cases infected persons may suffer with watery diarrhoea initially but is progressed to bloody diarrhoea in some cases with in one to two days leads to haemorrhagic colitis [65]. Also frequently reported the severe abdominal pain. In some cases infection progresses to haemolytic Uremic Syndrome (HUS) and Thrombocytopenia [39].
Since last 50 years, several antibiotics have been used in the treatment of common bacterial infection in both human and veterinary medicine [75] and also used as growth promoters in poultry production [49]. Because of over use and misuse of antibiotics in the treatment of infections is considered as one of the important factor in the development of antimicrobial resistance [35]. Antimicrobial resistance genes are present either on the chromosome or on the mobile genetic elements (i.e., plamids) in the antibiotic resistance isolates [1]. These plasmids transfer via conjugation among the bacterial population [38], which may entered in to the food chain. Antibiotic resistance limits the treatment options [42] and prolongs the disease process. It become a dangerous zoonotic pathogen could affect the epidemiology of *E. coli* in human beings.

Thus the development of antimicrobial resistance is a concern not only to a veterinarians but also to public health. Therefore, the present study was designed to isolate *E. coli* strains from dairy farm sewage in and around Tirupati, Andhra Pradesh, India for assessing their susceptibility and resistance patterns to some selected antimicrobial agents.

Materials & Methods

Collection of Samples

The specimens selected for this study were dairy farm sewage and wastewater. The sewage and wastewater samples were collected from different organized and unorganized dairy farms and animal sheds in and around Tirupati, aseptically in sterilized plastic containers. A total of 128 samples from different sources viz: Organized dairy farms (n=28), unorganized dairy farms (n=32) and animal sheds (n=68) were collected aseptically in sterilized plastic containers. The collected specimens were processed within 2 to 24 hours of collection.

Isolation and Identificatio

Tryptone soy broth (TSB) was used for enrichment of inoculum and incubated at 37 ^{0}C for 24h. After overnight incubation, the cultures were streaked on MacConkey agar and Eosine Methylene Blue (EMB) agar plates and the plates were incubated at 37 ^{0}C for 24h. After incubation the plates were observed for lactose fermenting colonies and greenish metallic sheen colonies respectively. The colonies thus obtained were transferred to nutrient agar slants in duplicate and incubated at 37 ^{0}C for 24 h and stored at 4^{0}C for further identification. Identification of the isolates was carried out by making smears and subjected them for Gram's method of staining for identification of the organisms. For confirmation of *Escherichia coli*, the biochemical tests conducted were triple sugar iron agar test, urease test, motility test and IMViC tests.

Antimicrobial Susceptibility Test

The modified disc diffusion method of Bauer *et al.* [14] was employed and the interpretation was made as per the interpretation chart provided by the manufacturer using panel of 14 antibiotics.

For antibiotic sensitivity testing of isolates the inoculum was prepared by transferring 4-5 colonies from primary isolated medium i.e. MacConkey agar and EMB agar plates to 5 ml of Tryptic soya broth by touching the top of the colonies with a flame sterilized and cooled platinum loop and incubated the bacterial suspension at 37^{0}C for 8 h. After incubation, the resulted culture was compared with the turbidity standard prepared separately for adjustment of bacterial suspension.

The turbidity standards were prepared by adding 0.5 ml of (1.17% w/v) of Barium chloride dehydrate ($BaCl_2$ $2H_2O$) solution to 1% Sulphuric acid. The turbidity standard was placed in the tube identical to the one used for the broth sample and was stored in the dark at room temperature. The turbidity was equivalent to 10^8 CFU/ml which is half the density of a Mac Farland 0.5 standard. The standard was agitated on a vortex mixer immediately before use. If the culture was found less turbidity than the turbidity standard, it was further incubated for 2-8 h at 37^{0}C until turbidity was equivalent to the standard. If the turbidity exceeds that of the standard the culture solution was diluted with tryptic soya broth to equitate with the standard.

Commercially available standard antimicrobial discs (Hi-Media) were procured and stored at 2-8 ^{0}C in the refrigerator. Unopened disc containers were removed from the refrigerator 1-2 h before use, to bring them to room temperature. The antimicrobial discs with known concentrations as noted in micrograms (µg) or International Units (I.U) per disc were used to study the antimicrobial susceptibility of the isolates. The antimicrobial discs used in this study are given in Table 1.

Muller-Hinton (MH) agar, the recommended medium for disc diffusion test was used in the present study. Medium was prepared according to protocol provided by manufacturer and autoclaved at 121 ^{0}C, 15 lbs for 15 min. When the temperature of the medium was reached between 45-50 ^{0}C it was mixed well and approximately 15-20 ml of medium was added to the sterilized petriplates and incubated overnight at 37^{0}C for sterility testing and the uncontaminated plates were wrapped with aluminium foil and they were stored at 4 ^{0}C till use.

Inoculum for the antibiotic sensitivity test was prepared by using sterile swabs. The sterile cotton swab was dipped in the standardized bacterial suspension and rotated several times. Then the cotton swab was gently pressed on the upper inside wall of the test tube to remove excess inoculum. The swab was then streaked over the entire surface of the MH agar plate for three times. The plate was turned at 60^0 angle between each streak to ensure even distribution of the inoculum. A final sweep of the swab was made around the agar rim.

Allowed the inoculated plates to dry for 5 to 15 min and placed the selected antimicrobial discs with a distance of 24 mm apart by using a disc dispenser and gently pressed down on to the agar surface to provide uniform contact. The inoculated plates were inverted and incubated at 37 ^{0}C for 24-48 h. Each plate was examined after incubation for the diameter of zones of complete inhibition including the diameter of the discs were measured up to the nearest whole millimetre with ruler in non-reflecting background. The zone margin was the area where no obvious growth was visible and the readings were compared with that specified readings in the interpretive chart supplied by the manufacturer of the antibiotic discs and the results were documented as sensitive (S), intermediate resistant (I) and resistant (R) (Table. 1).

Results & Discussion

Among the 128 isolates no isolate was completely sensitive to any of the antibiotic test discs used in this study. Muller Hinton agar plates showing the sensitivity, intermediate sensitivity and the resistance patterns of various antibiotic discs were shown in Fig.1 and 2. Maximum resistance was observed for ampicillin (92.19%), Penicillin-G (76.56%), cefotaxime (71.10%), streptomycin (64.84%), gentamicin (64.06%), chloramphenicol (57.81%), tetracycline (54.69%), cefoperazone (51.56%), ofloxacin (44.53%), cefadroxil (39.06%), ciprofloxacin (38.28%), azithromycin (30.40%), meropenem (27.34%), and tigecycline (20.31%) (Table 2 & Fig. 3).

E. coli isolated in this study was highly resistant to ampicillin (92.19%). The results are comparable with Anago *et al.*(2015) [9] and Atere *et al.* (2015)[13], who observed 97.6% and 89.6% of resistance respectively to ampicillin by the *E. coli* isolates, Niranjan and Malini (2014)[52] observed 88.4% of resistance to ampicillin whereas Arabi and Banazadehi (2013)[10] and El-Rahman *et al.* (2017)[26] observed the antibiotic resistance pattern of *E. coli* isolates and their results showed that 100% of isolates were resistance to ampicillin, A little lesser

resistance to ampicillin than in the present investigation was observed by Nitika *et al.* (2014)[53] and Mustika *et al.* (2015)[50] who reported 81.4 % and 80.0% respectively, and in contrast to the results obtained in this study Adenaike *et al.* (2016)[3], Zinnah *et al.* (2008)[76], Eryulmaz *et al.* (2010)[28], Bonnedahl *et al.* (2015)[18], Melo *et al*. (2015)[47] and Aasmae *et al*. (2015)[2], reported 69%, 59%, 56%, 30.1%, 20.2% and 4.4% of resistance among the *E. coli* isolates to ampicillin respectively. Whereas Goncouglu *et al* (2010)[30] reported 0% of resistance among the *E. coli* isolates to ampicillin.

In the present study 76.56% resistance was observed for Penicillin-G and higher resistance of 100% than the present study was reported by Sabir *et al.* (2014)[67], Nontongana *et al.* (2014)[54], Maloo *et al.* (2014)[43] and Mustika *et al.* (2015)[50]. Jeyasanta *et al.* (2012)[36], who also reported a little higher resistance of 82.41% to penicillin-G whereas low resistance (63%) was reported by Chandrasekaran *et al.* (2014)[22].

The *E. coli* isolates in this study exhibited 3s9.06% resistance to cefadroxil. Higher resistance by *E. coli* isolates for this antibiotic was observed by Khan *et al.* (2014)[40] who observed 97.62% of resistance, Kumar *et al.* (2013)[41] reported 88.52% of resistance, Rahim *et al.* (2014)[60] observed 85.71% of resistance and Mishra *et al.* (2013)[48] observed 58.33% of resistance to cefadroxil, whereas Bonnedahl *et al.* (2015)[18] and Sundvall *et al.* (2014)[71] analysed the antibiotic resistance pattern of pathogenic *E. coli* and reported that only 15.1 % and 2.6% of isolates were resistant to cefadroxil respectively.

The resistance to cefotaxime by *E. coli* isolates was 71.10% in the present study. Similar to the present investigation Ranjini *et al*. (2015)[62] reported 71.42% of resistance to cefotaxime. Very high resistance to cefotaxime was observed by Kumar *et al.* (2013)[41], who reported 90.16% of resistance, Arabi and Banazadehi (2013)[10] who found 81.9% of resistance, when compared to the results obtained in the present investigation. Hussain *et al.* (2015)[33] observed 67% of resistance, Raihan *et al.* (2014)[61] observed 60% of resistance, Ali *et al.* (2014)[5] reported 58.5%, Manikandan and Amsath (2014)[44] found 58% of resistance, Anago *et al.* (2015)[9] reported 56.5% of resistance, Ferdosi *et al*. (2015)[29] found 45.6% of resistance, El-Rahman *et al*. (2017)[26] who observed 40% of resistance, Sohail *et al.* (2015)[69] found 29.50% of resistance, which are less than the present study. Melo *et al*. (2015)[47] reported very low resistance (1.2%) to cefotaxime by *E. coli* isolates.

E. coli isolates in the present study have shown 51.56 % of resistance to cefoperazone. Saeed *et al.* (2009)[68] observed 65.5% of resistance, Mishra *et al.* (2013)[48] observed 66.66% of resistance and Ranjini *et al.* (2015)[62] revealed 75.97% of resistance to cefoperazone among the *E. coli* isolates, which are higher than the present study, On the contrary Sohail *et al.* (2015)[69] reported only 29.50% resistance, Asati (2013)[12] observed 21% of resistance and Tanvir *et al.* (2012)[72] reported 13.2% of resistance by the *E. coli* isolates towards cefoperazone.sss

In the present investigation the *E. coli* isolates have shown 27.43% of resistance to the antibiotic meropenem. Similar to these studies a lower resistance to meropenem than in the present study was observed by Nitika *et al.* (2014)[53] who reported 25.4% of resistance, and Sohail *et al.* (2015)[69] reported 1.22% of resistance to meropenem whereas Mishra *et al.* (2013)[48] found 41.66% of resistance, Vij *et al.* (2014)[73] observed 62.7% of resistance and Biswas *et al.* (2014)[16] who observed 100% resistance to meropenem by the *E. coli* isolates, which was considerably higher than the resistance observed in the present investigation. On contrary Akter *et al*. (2016)[4] reported zero (0%) per cent resistance to meropenem by the *E. coli* isolates.

In the present study 64.06% of resistance was observed for gentamicin by the isolates. Higher resistance to gentamycin than in the present investigation was observed by Biswas *et al.* (2014)[16] who observed 94.11% of resistance, Arabi and Banazadehi (2013)[10] found 82.5% of resistance, Rawat *et al.* (2010)[63] reported 70.86% of resistance and Atere *et al.* (2015)[13] found a little higher resistance (68.8%) among the *E. coli* isolates, whereas Manikandan and Amsath (2014)[44] found 62.5% of resistance which is almost similar to the present study. Comparatively lower resistance to gentamicin than in the present study was observed by Ranjini *et al.* (2015)[62] who reported 56.98% of resistance, Anago *et al.* (2015)[9] found 45.2% of resistance, Ferdosi *et al.* (2015)[29] observed 36.8% of resistance, Zinnah *et al.* (2008)[76] reported that 32% of resistance, Sohail *et al.* (2015)[69] revealed that 19.26% of resistance, Alshara (2011)[6] found 17.3% of resistance, El-Rahman *et al.* (2017)[26] reported 10% of resistance, Eryulmaz *et al.* (2010)[28] observed 9% of resistance and Ali *et al.* (2014)[5] who reported 5% of resistance among the *E. coli* isolates to gentamycin whereas 0% resistance to gentamycin was observed by Goncouglu *et al* (2010)[30] and Akter *et al*. (2016)[4].

Habrun *et al.* (2010)[31] observed the antimicrobial sensitivity of *E. coli* isolated from the different organs of pigs in breeding farm and reported that 91% of the isolates were resistance to streptomycin and Stephan and Schumacher (2001)[70] found only 17.07% of susceptibility to streptomycin, whereas in the present study resistance to streptomycin was observed only in 64.84% of the isolates. Higher resistance than the isolates of present investigation was observed by Cergole-Novella *et al.* (2011) [21] who reported 78.1% of resistance and Saeed *et al.* (2009)[68] observed 72.4% of resistance to streptomycin by the *E. coli* isolates. Pyatov *et al.* (2014)[59] reported 48.6% resistance, Daniel *et al.* (2012)[24] observed 59.0%, whereas very low resistance levels viz. 30.0%, 20%, 11.0%, 9.09% and 4.4% were reported by Sabir *et al.* (2014)[67], Mustika *et al.* (2015)[50], El-Shatoury *et al*. (2015)[27], Goncouglu *et al* (2010)[30] and Aasmae *et al.* (2015)[2] respectively.

The resistance of ciprofloxacin in the present study was 38.28%. The resistance to ciprofloxacin in the present investigation was lower than the resistance reported by Anago *et al.* (2015)[9], Biswas *et al.* (2014)[16], Ranjini *et al.* (2015)[62], Arabi and Banazadehi (2013)[10], Ohieku and Magaji (2013)[56], Kumar *et al.* (2013)[41] and Atere *et al.* (2015)[13] who reported 91.7%, 88.23%, 84.91%, 78%, 58%, 54.10% and 47.9% respectively, whereas lower resistance of 33.60%, 24.6%, 15%, 14.5%, 10.6%, 10%, 8%, 4.7% and 2.9% was reported by Sohail *et al*. (2015)[69], Ferdosi *et al.* (2015)[29], Eryulmaz *et al.* (2010)[28], Alshara (2011)[6], Aminu and David (2015)[8], El-Rahman *et al.* (2017)[26], Zinnah *et al.* (2008)[76], Melo *et al.* (2015)[47] and Aasmae *et al.* (2015)[2] respectively. On the contrary Goncouglu *et al* (2010)[30], Raihan *et al.* (2014)[61], El-Shatoury *et al.* (2015)[27] and Adenaike *et al.* (2016)[3] have showed 0% (zero) resistance to ciprofloxacin by the *E. coli* isolates.

In the present investigation the *E. coli* isolates have shown 44.53% of resistance to ofloxacin. Higher than the resistance that was observed in this study was reported by Mary and Usha (2013)[46] who observed 97% of resistance, Mishra, *et*

al. (2013)[48] found 83.33% of resistance, Ohieku and Magaji (2013)[56] observed 71% of resistance, Manikandan and Amsath (2014)[44] observed 64.5% of resistance, Ibrahim *et al.* (2012)[34] reported that 55.1% of resistance and Atere *et al.* (2015)[13] reported 52.1% resistance to ofloxacin among the *E. coli* isolates. Whereas very little resistance than in the present study was observed by Ferdosi *et al.* (2015) [29] and Oluyege *et al.* (2015) [57] who reported 8.8% and 3.9% of resistance respectively.

The antibiotic azithromycin has shown 30.40% of resistance among the *E. coli* isolates in the present investigation. Higher resistance to azithromycin by *E. coli* was reported by Raihan *et al.* (2014)[61] who found 100% of resistance, Aminu and David (2015)[8] observed 76.6% of resistance, Pant *et al.* (2015)[58] observed 71.0% resistance, Chayani *et al.* (2009)[23] have shown 60.37% of resistance, Zinnah *et al.* (2008)[76] observed 33% of resistance. Aly *et al.* (2012)[7] revealed 31% of resistance, which was similar to the present findings for various clinical samples, whereas Akter *et al.* (2016)[4] reported only 11.0% resistance in their *E. coli* isolates.

In the present investigation chloramphenicol has shown 57.81% of resistance among the *E. coli* isolates whereas higher resistance to chloramphenicol by *E. coli* isolates was reported by Pant *et al.* (2015)[58] who reported 70% of resistance and Rawat *et al.* (2010)[63] found 61.14% of resistance. Saeed *et al.* (2009)[68] observed 58.6% of resistance, which was almost similar to the present findings, whereas Ibrahim, *et al.* (2012)[34] and Melo *et al.* (2015) [47] reported 22.4% and 4.7% of resistance respectively, which are lower than the present study in *E. coli* isolates of various sources. Whereas Goncouglu *et al* (2010) [30] and Joshi *et al* (2012) [37] who reported 0% resistance to chloramphenicol.

The resistance to tetracycline was 54.69% in this study. Adenaike *et al.* (2016) [3] reported 54.0% resistance, which is similar to the present findings, whereas lower resistance of 40.4% and 26% were reported by Aminu and David (2015) [8] and Melo *et al.* (2015)[47] respectively. Very low resistance of 7.0%, 2.9% and 0% were reported by El-Shatoury *et al.* (2015) [27] and Aasmae *et al.* (2015)[2] and Goncouglu *et al* (2010) [30] respectively. Higher resistance of 100% (Pant *et al.*, 2015 [68] and Nsofor *et al., 2016* [55], 88.2% (Oluyege *et al.,2015* [57] 77.1% (Ibrahim *et al.,2012*) [34], 69.4% (Sabir *et al.,2014*) [67] and 60% (Zinnah *et al.*, 2008) [76] than the present study for the *E. coli* isolates obtained from different sources.

E. coli isolates in the present study have shown 20.31% of resistance to the antibiotic tigecycline. Higher resistance than in the present study was observed by Mantzourani *et al.* (2015) [45] who found 100% resistance by the *E. coli* isolates, whereas Ali *et al.* (2014) [5] reported only 2.5% of resistance to tigecycline, on the contrary Nandi *et al.* (2014) [51], Behera *et al.* (2009) [15] and Rossi *et al.* (2008) [66] have reported 100% sensitivity to tigecycline by the *E. coli* isolates.

Although it is extremely difficult to explain these conflicting data with regards to both time and place of study, the variation is probably due to differential clonal expression and drug pressure in community.

Table 1: Antimicrobial discs used to study the antimicrobial susceptibility of the isolates

S.No	Name of the antimicrobial disc	Quantity of antimicrobial substance per disc	Diameter of zone of inhibition in mm (as per the manufacturer guidelines)		
			Sensitive	Intermediate	Resistant
1	Ampicillin	10 mcg	≥ 17	14-16	≤ 13
2	Gentamycin	10 mcg	≥ 17	13-14	≤ 12
3	Streptomycin	10 mcg	≥ 15	12-14	≤ 11
4	Ciprofloxacin	05 mcg	≥ 21	16-20	≤ 15
5	Chloramphenicol	30 mcg	≥ 18	13-17	≤ 12
6	Penicillin-G	10 units	≥ 29	---	≤ 28
7	Cefoperazone	75 mcg	≥ 21	16-20	≤ 15
8	Cefotaxime	30 mcg	≥ 23	15-22	≤ 14
9	Tigecycline	15 mcg	≥ 16	13-15	≤ 12
10	Meropenem	10 mcg	≥ 23	20-22	≤ 19
11	Azithromycin	15 mcg	≥ 18	14-17	≤ 13
12	Cefadroxil	15 mcg	≥ 18	15-17	≤ 14
13	Ofloxacin	05 mcg	≥ 16	13-15	≤ 12
14	Tetracycline	30 mcg	≥ 19	15-18	≤ 14

Table 2: Antimicrobial sensitivity/intermediate/resistant-patterns of *Escherichia coli* from different sources

S. No	Antimicrobial agent	Pattern of antibiogram		
		Sensitive (%)	Intermediate (%)	Resistant (%)
1	Ampicillin	06 (4.68%)	04 (3.13%)	118 (92.19%)
2	Gentamycin	24 (18.75%)	12 (9.37%)	82 (64.06%)
3	Streptomycin	23 (17.96%)	12 (9.37%)	83 (64.84%)
4	Ciprofloxacin	63 (49.22%)	16 (12.50%)	49 (38.28%)
5	Chloramphenicol	31 (24.22%)	23 (17.97%)	74 (57.81%)
6	Penicillin-G	30 (23.44%)	00 (0.00%)	98 (76.56%)
7	Cefoperazone	48 (37.50%)	14 (10.94%)	66 (51.56%)
8	Cefotaxime	20 (15.62%)	17 (13.28%)	91 (71.09%)
9	Tigecycline	91 (71.10%)	11 (8.59%)	26 (20.31%)
10	Meropenem	76 (59.37%)	17 (13.28%)	35 (27.34%)
11	Azithromycin	63 (49.22%)	29 (22.65%)	38 (29.69%)
12	Cefadroxil	44 (34.37%)	34 (26.56%)	50 (39.06%)
13	Ofloxacin	45 (35.15%)	26 (20.31%)	57 (44.53%)
14	Tetracycline	36 (28.12%)	22 (17.19%)	70 (54.69%)

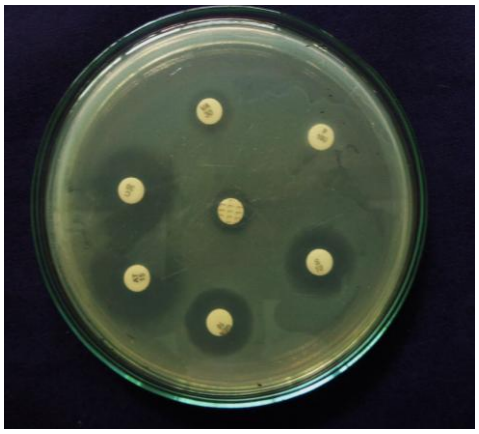

Fig 1: Antibiotic sensitivity and resistance pattern of the *E. coli* isolates (Ampicillin, Azithromycin, Chloramphenicol, Gentamicin, Streptomycin, Penicillin-G and Tetracycline)

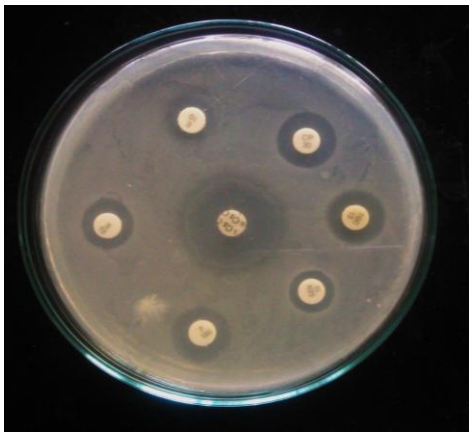

Fig 2: Antibiotic sensitivity and resistance pattern of the *E. coli* isolates (Ciprofloxacin, Meropenem, Tigecycline, Cefadroxil, Cefotaxime, Cefoperazone and Ofloxacin)

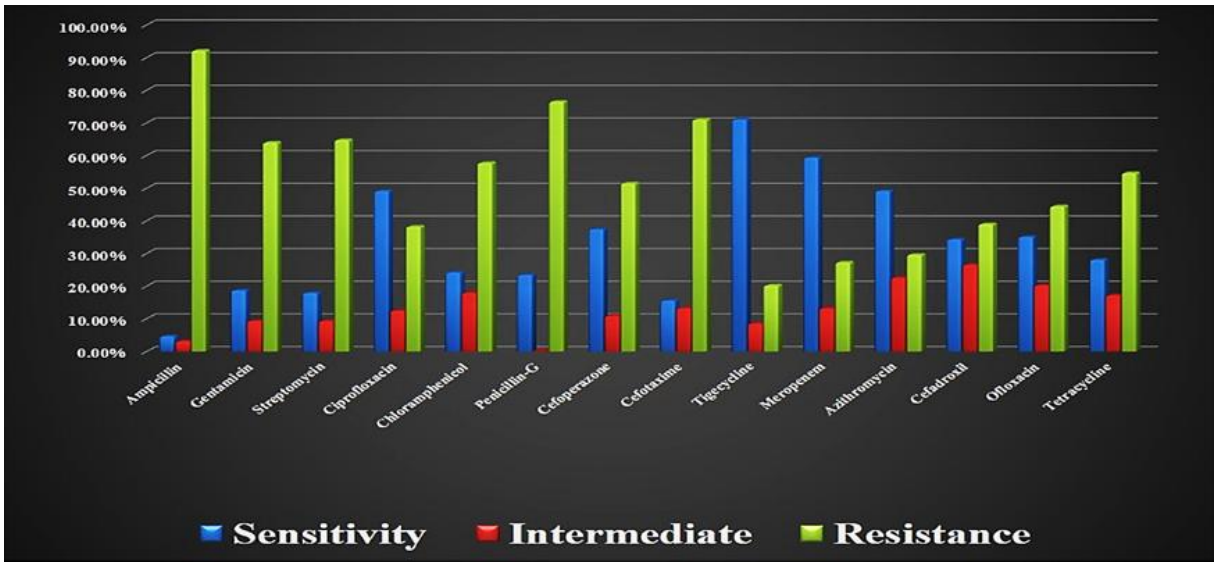

Fig 3: Antimicrobial Sensitivity/ Intermediate/ Resistance patterns of *E. coli* from different sources

Conclusion

the misuse and over use of antimicrobials is accelerating the development of antimicrobial resistant strains. Antimicrobial resistance is a complex problem that effects all of society and is driven by many interconnected factors. Single, isolated interventions have limited impact. Coordinated action is required to minimise the emergence of antimicrobial resistance.

Authors contribution

AJB supervised the work. The manuscript was written by SSSG, finally revised by AJB. All authors read and approved the final manuscript.

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