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Antimicrobial drug resistance of *E.coli* isolated from different soil samples in and around Thiruvarur district, Tamilnadu, India

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Abstract
Most antibiotics are used today are isolated and extracted from microbial source. The emergence of antibiotic resistance and need for better, broad spectrum antibiotics is always in high demand. Hence the present study was conducted to determine antibiotic sensitivity pattern of E.coli isolated from composite soil sample and identified by using the biochemical tests. Totally 25 antibiotics were used for the detection of antibiotic resistance profile of E.coli, antibiotics namely Ampicillin, Cephalothin, Streptomycin, Nitrofurantoin, Tetracycline, Chloromphenical, Amoxycillin clavunalic acid, Cefipirome, Cefpodoxime, Cefataxin, Nalidixic acid, Gentamycin, clindamycin, Doxycycline, Cefazolin, Levofloxacin, Meropenam, Lincomycin, Tobramycin, Vancomycin, Amoxyclave, Cephalexin, Norfloxacin, Colistin, Lemofloxacin. Antibiotic resistance was done by Kirby Bauer method.Ciprofloxacin and Vancomycin has showed the highest Zone of inhibition nearly 98%, After primary screening of antibiotic resistance *E.coli* showed antimicrobial activity against some bacteria and fungi namely *Staphlococcus aureus (5.0mm), Pseudomonas aeruginosa(3.9mm), Klebsiella pneumonia(8.2mm), Pencilliumchrysogenum (12.5mm), Aspergillus species.(10.0mm),* The results showed that *Escherichia coli* was commensal organism and exhibited multidrug resistances, necessitating efficient antibiotic stewardship guidelines to streamline their use in the production industry.

Keywords: composite soil, antibiotics, antimicrobial activity, antibacterial resistance

Introduction
Escherichia coli is the most prevalent facultative anaerobic species in the gastrointestinal tract of human and animals, usually a harmless microbe, but it is also a medically important bacteria causing a number of significant illnesses.(Friedman *et al* 2002) [7, 11]. It has been suggested that resistance in bacterial populations may spread from one ecosystem to another (Johnson 2007) [5]. The wild dissemination of antimicrobial resistance among bacterial populations is an increasing problem worldwide.
Antibiotics are often used for therapy of infected humans and animals as well as for prophylaxis and growth promotion of food producing animals. Many findings suggest that inadequate selection and abuse of antimicrobials may lead to resistance in various bacteria and make the treatment of bacterial infections more difficult (Kolar 2001). Antimicrobial resistance in *E. coli* has been reported worldwide. Treatment for *E. coli* infection has been increasingly complicated by the emergence of resistance to most first-line antimicrobial agents (Sabate 2008) [17] Over the years, resistance to cephalosporins among members of enterobacteriaceae has increased mainly due to the spreading of Extended-spectrum β-Lactamases (Yusha'u 2010) [20].

Antibiotic resistance
Antibiotic resistance happens when the germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow. Infection caused by antibiotic resistant germs is difficult, and sometimes impossible to treat.

The aim of the study is to determine the antimicrobial susceptibility of antibiotic resistance *E.coli* isolated from different soil samples in and around Thanjavur district, Tamilnadu.

Materials and Methods
Sample Collection
Soil sample were collected to a depth of approximately 5cm from botanical garden, agricultural waste, poultry farm soil, pond soil at Thiruvarur district, Tamilnadu, India. At each location mixed composite soil samples were taken several feet apart to account for variation in soil. The collected soil samples were sealed in sterile polythene bags and stored at 4˚C to prevent the bacterial death. The soil sample was sieved to extract from fine soil particles which were then serially diluted for isolating microorganism's that are potential antibiotic producers.

Isolation of Microorganism from Soil: Dilution (Aneja, 1996) [1]
Serial dilution was performed by using the collected sample to isolate the bacteria. 1 gram of soil sample was diluted in the tube containing 9 ml of sterile distilled water and mixed thoroughly to make 1:10 (10^2). 1 ml of transferred to the next test tube and serially diluted in to the series of test tube having 9 ml of sterile pipettes upto10^7 dilution.
For the isolation of bacteria, nutrient agar medium was sterilized at 121˚ C for 15 minutes, pH (6.5 ± 0.2). Petriplates were sterilized and labelled properly. 1 ml of sample from, $10^5, 10^6$, dilution was transferred into the respective plates. The plates were incubated under anaerobic

condition at 30˚C for 48 – 72 hours and the colonies were counted. Different colonies were observed and transferred to other specific media for identification.

Isolation and screening and Identification of Microbial Isolates

By using soil sprinkle technique, five different bacterial colonies having zone of inhibition were picked and streaked on separate nutrient agar plates to get pure Cultures. out of 5 bacterial isolates only one bacterial isolate showed antibiotic producing ability and identified as *E.coli.*

Identification of Bacteria

GRAM'S Staining (Han's Christian Gram, 1884)

The colonies on the plates were subjected to staining method, in order to identify the morphology and gram reaction of the bacterium. A thin smear was prepared on a clean slide using the isolated individual colony. The smear was heat fixed and cooled. The dried smear was then flooded with the primary stain, crystal violet and allowed to stand for one minutes. Then slide was then washed with water and decolourized with 95% of ethanol, for a few seconds and gently with running tap water. Then the slide was flooded with a counter stain, safranin for one minute. After drying, the stained smear was observed under microscope to identify the organisms.

Most bacteria can be differentiated by their gram's reaction due to differences in their cell wall structure. The organisms which stained dark purple with crystal violet (primary stain) and not decolourized by 95 % alcohol were referred as gram's positive and those organisms which after being stained with crystal violet lose their colour when treated with alcohol and stained red with safranin (counter stain) were referred as gram's negative.

Motility Test (Bailey and Scott., 1996)

The motility was done by the employing hanging drop method. A drop of culture broth was placed on the centre of the coverslip. Vaseline was applied over each corner of the cover slip. Then the slide was observed through the oil immersion microscope.

Biochemical Tests (Norris and Ribbon, 1972)

Indole Test

1% Tryptone broth was prepared and sterilized in the autoclave at 15psi (121˚c) for 15 minutes. The tryptone broth was inoculated with microbial of the desired colony of the plates. Tubes were incubated at 35˚ c for 24 to 48 hours. Presence of cherry red ring formation at top layers of the samples in the tubes incubates positive result. Absence of cherry red ring formation at formation at layer of the samples in the tubes indicates negative results.

Methyl–Red Test

Methyl red broth was prepared and sterilized in the autoclave at 15 psi (121˚c) for 15 minutes and broth was poured into test tubes and inoculated with bacterial culture and then incubated at 37˚c for 24 to 48 hours. After incubation 5 drops of methyl red was added and change in colour indicates negative result.

Voges–Proskauer Test

Voges-proskauer broth was prepared and sterilized in the autoclave at 15 psi (121˚c) for 15 minutes and poured into the test tubes and inoculated with bacterial culture and incubated at 37˚c for 25 to 35 minutes to complete the reactions. Then the formation of pink red colour in the medium indicates the positive test and the formation of faint brown colour indicate the negative test.

Citrate Utilization Test

Simmon's citrate agar slant was prepared. All the constituents were dissolved, except phosphate, which was dissolved separately in 100 ml distilled water and volume made up to 1 litre. pH was adjusted to 6 – 9 and the medium was poured in the culture tubes and sterilized in the autoclave at 15 psi (121˚c) for 15 minutes and the slants were inoculated with particular organism isolated from the soil. All the slants were incubated at 37˚c for 48 hours. In the citrate utilization test, the development of blue colour indicates the positive result for citrate, while turning of blue to green indicate negative result.

Catalase Test

Trypticase soy agar slants were prepared. All the constituents were dissolved in 1000 ml distilled water and it was poured into sterile test tubes. The medium was sterilized by autoclavingat 151bs pressure for 15 minutes and slants were prepared. The trypticase soy agar slant were inoculated with particular colonies. All the slants were incubated at 37˚ c for 48 hours. Few drops of H_2O_2 were added to the slants which from bubbles formation indicates the negative test.

Oxidase Test

Nutrient agar medium were prepared, sterilized and poured into the petri plates. The isolated were added to the surface of the inoculated plates. A filter paper disc (contain freshly prepared 1% NNN- tetraethyl para-phenylenediamine dichloride) was place on incubated plates served for the formation of the purple colour within 30 seconds in the oxidase disc confirmed the oxidase activity of the organism. The absence of colour formation showed a negative oxidase activity of the organism.

Urease Test

Christensen's urea agar medium was prepared, sterilized and poured into a test tube. The bacterial culture was streaked on surface of the slant culture and the tube was observed for the colour change from yellow to pink. No colour change in medium shows negative reaction.

Subculturiung of Microorganisms

Bacterial colonies is the clear margin was picked and subculture on fresh nutrient agar plates using sterile loop, using streak plate method in laminar air flow to purify. The isolates followed by incubation for 24 hours at 37˚C.

Crowded plate technique for isolation and screening antibiotic producing *e.coli* Microorganisms

One gram of soil sample was weighed and mixed in 10 ml of sterile distilled water to get 1:10 dilution, then thoroughly mixed by vigorous shaking. After allowing the sediment to settle, supernatant was used for subsequent dilutions. Dilution were prepared by taking 1ml of stock solution and transferring into 9 ml of sterile distilled water in another test tube to give 1:100. This process of transfer from proceeding tube continued till 1:10000 dilution was archived 0.1 ml of

soil inoculums from each dilution was taken and inoculated separately onto petri plates within nutrient agar media of pH 7- 7.2. Plates were incubated at room temperature for 2 days in inverted position. Colonies that produced zone of clearance were sub cultured in nutrient broth (Hi – Media, India) and their pure cultures were stored in 4˚c until further use.

Sources of Antibiotics

The antibiotics were used for this study namely, Ampicillin, Cephalothin, Streptomycin, Nitrofurantoin, Tetracycline, Chloromphenical, AmoxycillinClavulanic acid, Cefpirome, Cefpodoxime, cefphotaxaime, Nalidixic acid, Gentamycin, Clindamycin, Doxycycline, Cefazolin, Levofloxacin, Lincomycin, Tobramycin, Vancomycin, Amoxyclav, Cephalexin, Norfloxacin, Colistin, Lemofloxacin, Meropenam were procured from a renowned store at Madras scientific supplies, Trichy.

Antibiotic Susceptiblity Test (H.Jan, 2013)

Antimicrobial sensitivity test, through Kirby diffusion test, was performed for all *E.coli* isolates following the protocol. At least 4-5 well isolated colonies of the same morphological type are selected from a non-selective agar plate (nutrient agar); just the top of the colonies is touched and the growth transferred to a tube containing 4-5ml NSS or an equivalent medium such as peptone water broth. The inoculated broth is incubated at 35˚-37˚c until a slight visible turbidity appears, usually within 22-28 hours. The turbidity of the pre incubated broth and the suspension of bacteria are adjusted by comparisons with 0.5Mc far land turbidity standards. The standard and the test suspension is adjusted with broth or saline compared with turbidity standard against a white background with contrasting black lines, until the turbidity of the test suspension equals the turbidity standards. The bacterial suspension was inoculated on to Muller Hinton agar (Oxide UK) with sterile swab cover the whole surface of the agar. The inoculated plates were left at room temperature to dry. Before using the antimicrobial discs, they were kept at room temperature for one hour and then dispended on the surface of media. Following this the plates were incubated aerobically at 37˚C for 24 hours. The diameter of the zone of inhibition around the disk were measured to the nearest millimetre using calibrate rulers, and the isolates were classified as susceptible, intermediate, and the resistant according to the interpretative accordance with the antibiotics, namely Chloramphenicol(5mcg), Cephalothin (3mcg), Amoxyclav (6mcg), Vancomycin (4mcg), Nitrofurantoin (3mcg), Levofloxacin (5mcg), Nalidixic acid (4mcg), Tetracycline (3mcg), Meropenam (2mcg), Amoxicillin (3.0mcg), Ampicillin (5mcg), Doxycycline (4mcg), Clindamycin (5.0mcg), Colistin (4mcg), Ciprofloxacin (6mcg), Cefipirome (2.5mcg), Tobramycin 6.0mcg), Tetracycline (3.0mcg), Cefazolin (4.3mcg), Lincomycin 5.0mcg), Gentamycin (2.2mcg), Streptomycin (5.0mcg), cefixime (3.8mcg), Doxycycline (2.3mcg).

Antimicrobial activity by agar diffusion assay (ventola2015)

Agar well diffusion method was used to check the culture for the production of antimicrobial metabolites. Twenty four hours fresh culture of *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Penicillium chrysogenum, Aspergillus species,* were diluted with pre-sterilized normal saline. a sterilized cotton swab was dipped in the diluted cultures and lawn were prepared over the agar surface. Wells were made in the inoculated plates using sterile corn borer. About 80ul cell free supernatants were added in the wells, and the plates were incubated at 37˚C for 24 hours. After 24 hours, the zone of inhibition was observed. The diameter of the zone of inhibition was measured in (mm) with well size of 6mm.

Selected resistant *E.coli* culture was used for antibacterial activity testing by using Kirby Bauer disc diffusion method. Antibiotics resistance *E.coli* was used for antimicrobial activity of selected pathogens. The pathogens from procured from the ClinicalLaboratory. The selected test organism were namely, *Staphylococcus aureus, Klebsiella pneumonia Pseudomonas aeruginosa, Penicillium chrysogenum, Aspergillus species.*,used for study.

Statistical Analysis: (Gupta, 1977) [10]

Comparative resistant rate for antibiotics from the *E.Coli* were statistically analysed by comparative studies. Comparative antibiotics were analysed by standard deviation and mean deviation method.

All the experiment was reported as triplicates. The result obtained in the present investigation was subjected to statistical analysis like mean (X) deviation.

Mean (X) = sum of value of the variable

N = number of observation

Where, together x and x-. Divide the total by the number of observation. The Mean deviation
(MD) was calculated by formula. The formula for calculating

Mean deviation (MD) = € X – X-/N

Where, X = Number of all values
X_= arithmetic mean
N= total number of observation.

Results

E. coli is widely disseminated within the antibiotic-resistant community. In the present study, antibiotic producing bacteria were isolated from a soil sample. After primary screening, *E.coli* was isolated and showed antibiotic sensitivity against commercial antibiotics and antibacterial activity was tested against some selected bacterial and fungal pathogens. Totally 25 antibiotics were used for the detection of antibiotic resistance profile of *E.Coli*, antibiotics namely Ampicillin, Ceohalothin, Streptomycin, Nitrofurantoin, Tetracycline, Chloromphenical, AmoxycillinClavulanic acid, Cefpirome, Cefpodoxime, Cefataxin, Nalidixic acid, Gentamycin, Clindamycin, Doxycycline, Cefazolin, Levofloxacin, Lincomycin, Tobramycin, Vancomycin, Amoxyclav, Cephalexin, Norfloxacin, Colistin, Lemofloxacin, Meropenam were procured from a renowned store in Madras scientific supplies Trichy. (Figure- 6)

Isolation and screening of *e.coli*isolate

In the present study, soil sprinkle technique was used to isolate antibiotic producing *E.Coli*for this purpose about 20-

30 particles of soil were sprinkled on the surface of nutrient agar plates seeded with the test organism *E. coli*. The plates were incubated at 30˚C for 24 hours. Antibiotic activity was checked by zone of inhibition, surrounding a colony.

Identification of Antibiotic Producing *E.Coli*

Isolated bacterial strain was identified morphologically (shape, gram staining,) and biochemically shown the production of indole, citrate utilization, methyl red vogesproskauer (MR-VP), oxidase, catalase, nitrate reduction, gas production from glucose according to the Bergey's manual of determinative bacteriology.

Biochemical Characterization of Isolation and Identifiaction of Soil

Serial dilution technique was used to isolate the bacteria. Gram staining, motility test, and biochemical test were indole, MR-VP, citrate utilization, oxidase, catalse. The bacterial species are identified their morphological character, Biochemical and Bergey's manual of determinative bacteriology.

Identification of Bacteria

Gram negative, rod shaped, Non - Motile organism showed positive result for indole and methyl red test. Negative result for citrate test, mannitol, catalase, oxidase, VP

Antibiotic Susceptiblity Test

According to the stanndard operational procedures, antimicrobial susceptibility test were done on Muller Hinton Agar using Kirby Bauer disk diffusion method. The antimicrobial agents Over all resistance pattern of *E.coli* isolates showed Ciprofloxacin (20mm), Next,Vancomycin showed zone of inhibition vancomycin (14mm), followed by Gentamycin (15mm), Nalidixic acid (13mm), Ampicillin (11mm), Chloromphenical (09mm), Cefixime (09mm),

On the other hand, sensitivity profile of E.coli showed in Cephalothin (09mm), Next, Nitrofurantoin (07mm), Tobramycin (04mm), Followed by Cefalexin (03mm), Cefipirome (03mm), Cefazolin (02mm), Lincomycin (03mm), Norfloxacin (02mm).

The other antibiotics are showed Amoxyclav (05mm), Lemofloxacin (05mm), Meropenam (12mm), Doxycycline (05mm), colistin (08%), cefpodaxime (05mm),Clindamycin (19mm), Levofloxacin (08%) (Table-III) The tested antibiotics were Ciprofloxacin, (98%). chloromphenical, (98%), Vancomycin (98%), Nalidixic acid, (94%), Cephalexin, (86%) Amoxycillin (84%), Ampicillin (74%), Tetracycline (54%), Cefexime (36%), Gentamycin (54%). Highly sensitive to Cephalothin (56%), Nitrofurontoin (53%), tetracycline (50%), Amoxicillin (43%) Cefipirome (47%), Cefazolin (42%), Lincomycin (34%), Tobramycin (30%). moderately intermediate to Amoxyclav (21%), Lemofloxacin (20%), Meropenam (05%), Doxycycline (04%), Colistin (02%). Clindamycin (19%), Streptomycin (80%)

Antimicrobial Activity By Agar Diffusion Assay

Samples drawn during batch fermentations were subjected to agar well diffusion assay, using *Staphylococcus aureus*is a test organisms. Antimicrobial activity was measured in terms of zone of inhibition. The incubated samples were evaluated and optimum antimicrobial activity of inoculum of *E.coli* species was ensured at 48 hours.

It was observed that *Penicillium chrysogenum*,(12.0mm) showed maximum fungal activity against *E.coli*, followed by S*taphylococcus aureus, Pseudomonas aeruginosa, and Aspergillus*species.,with a zone of inhibition of 10.6 mm,7.3mm,8.0mm,13.0mm.(Table- I)

Statistical Analysis

E.coli was isolated showed significant zone of inhibition was observed at 48 hours of incubation. This indicates the antibiotic production microorganism to be stable aften 48 hours which read to be explored further.

In this present statistical analysis, chloromphenical, vancomycin, Nalidixic acid, amoxicillin, Ampicillin, Ciprofloxacin, tetracycline, cefixime, Gentamycin were highly resistance to the *E.Coli*, and followed by Cephalothin, Nitrofurantoin, Tetracycline, Doxycycline, Teicoplanin,, Cefipirome, Cefazolin, Lincomycin, Tobramycin were highly sensitive to the *E.coli*.. Another antibiotics of Lemofloxacin, streptomycin, Meropenam, Amoxyclav, colistin, cefixime, clindamycin were intermediate to the *E.coli*.

Bacteria are easy to isolate, culture, maintain and to improve their *E.coli* species being the predominant soil bacteria was selected for this study.

Totally 25 antibiotics were selected namely Chloromphenical(5mcg), Cephalothin(3mcg), Amoxyclav (6mcg), Vancomycin (4mcg), Nitrofurantoin (3mcg), Lemofloxacin (5mcg), Nalidixic acid (4mcg), Tetracycline (3mcg), Meropenam (2mcg), Amoxicillin (3.0mcg), Ampicillin (5mcg), Doxycycline(4mcg), Clindamycin (5.0mcg), Colistin (4mcg), Ciprofloxacin (6mcg), Cefipirome (2.5mcg), Tobramycin (6.0mcg), Cefpodaxime (3.0mcg), Cefazolin (4.3mcg), Lincomycin (5.0mcg), Gentamycin (2.2mcg), Streptomycin (5.0mcg), cefixime (3.8mcg), Doxycycline (2.3mcg). Moreover resistance pattern was showed the antibiotics such as Chloromphenical, vancomycin, Nalidixic acid, Amoxicillin, Ampicillin, Ciprofloxacin, Tetracycline,Cefixime, Gentamycin and sensitive pattern was showed on Cephalothin, Nitrofurantoin, Amoxicillin, Ampicillin, Cefipirome, Cefazoline, Lincomycin, Tobramycin. Antimicrobial activity was also done. *E.coli* showed better activity chloromphenical, followed by vancomycin. Inhibition was effectively control the pathogens on namely *Penicilliumchrysogenum (12.8mm)*, followed by *Staphylococcus aureus. (10.6mm).*

Table 1: Antimicrobial Activity of Antibiotic Resistant *E.Coli* Against Organisms

Test Organisms	Control(mm)	*E.Coli* (mm)
Staphylococcus aureus	0	5.0
Pseudomonas aeruginosa	0	3.9
Klebsiella pneumoniae	0	8.2
Aspergillus sps	0	10.0
Penicilliumsps	0	12.5

Table 2: Antibiotic Sensitivity Pattern

Antibiotics	Resistance (%)	Sensitive (%)	Intermediate (%)
Chloromphenical	49 (98%)	-	-
Cephalothin	-	46 (83%)	-
Amoxyclav	-	-	25(50%)
Vancomycin	49(98%)		-
Nitrofurantoin	-	42 (80%)	-
Lemofloxacin	-	-	31 (62%)
Nalidixic acid	47 (94%)	-	-
Tetracycline	27 (54%)	-	-
Meropenam	-	-	43 (86%)
Amoxicillin	42 (84%)	-	-
Ampicillin	37 (74%)	-	-
Doxycycline	-	-	20 (40%)
Colistin	-	-	28 (56%)
Ciprofloxacin	32 (64%)	-	-
Cefipirome	-	25(50%)	-
Tobramycin	-	13(26%)	-
Cefazolin	-	22(44%)	-
Clindamycin	-	-	20(40%)
Cefixime	18 (36%)	-	-
Lincomycin	-	30(60%)	-
Gentamycin	27 (54%)	-	-
Streptomycin	-	-	21(42%)
Levofloxacin	-	-	11 (22%).
Norfloxacin	-	10(20%)	-
Cefalexin	43 (86%)	-	-

Table 3: Statistical Analysis of Antibiotis Sensitivity

S.No	Antibiotics	Mean Deviation (MD)
1	Chloromphenical	28±12
2	Cephalothin	26 ±12.6
3	Amoxyclav	23 ± 7
4	Vancomycin	36± 10
5	Nitrofurantoin	23 ±12.3
6	Lemofloxacin	23±11.3
7	Nalidixic acid	28.3 ±12.3
8	Tetracycline	23 ±13
9	Meropenam	29 ±10
10	Amoxicillin	26 ± 10.3
11	Ampicillin	20 ±13
12	Doxycycline	2 0 ± 07
13	Colistin	27 ± 6.3
14	Ciprofloxacin	26 ±4.3
15	Cefipirome	22 ± 2
16	Tobramycin	26 ± 4.6
17	Cefazolin	24 ±4
18	Clindamycin	20.3 ± 0
19	Cefixime	17 ± 2.3
20	Lincomycin	19 ± 6.3
21	Gentamycin	18.3 ± 9.8
22	Streptomycin	23 ± 4
23	Levofloxacin	21 ±5
24	Norfloxacin	26 ±5
25	Cefalexin	14 ±9.3

Mean ± standard deviation

Statistical analysis of Kirby Bauer method

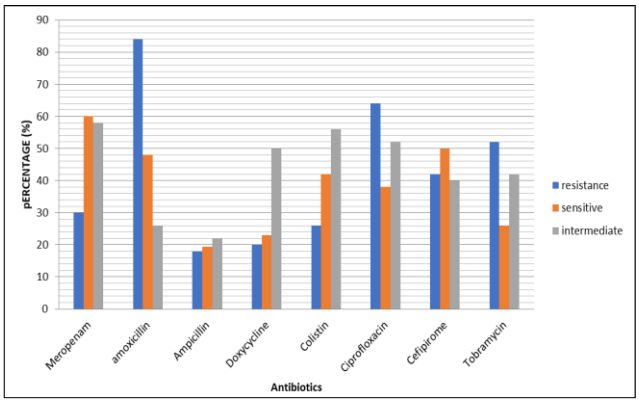

Fig 1

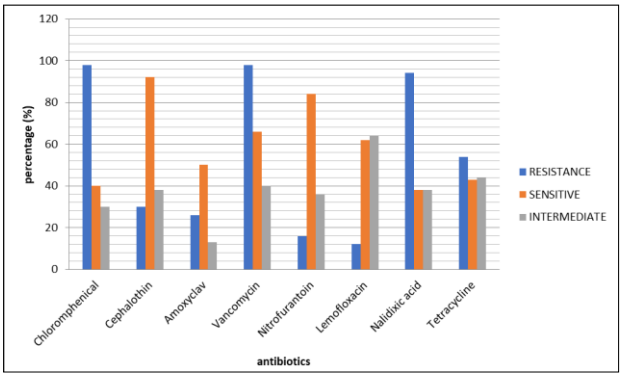

Fig 2

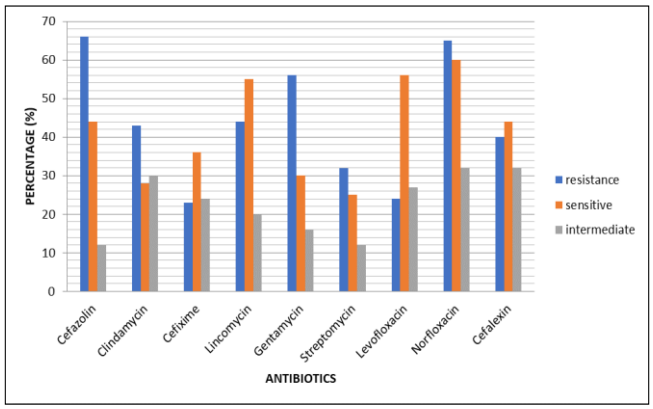

Fig 3

Our result was compared to (Cizman*et al.,* 2003) all e.coli isolates were resistant to atleast one antibiotics. Furthermore about 75.5% of bacterial isolate were resistant to more than two antibiotics (multi-drug resistant). Simultaneously, in our present study, used antibiotics were highly resistant to more than two antibiotics. Tetracycline is commonly used antibiotic against commensal E.Coli from chicken farms in two countries. In this present study the tetracycline was moderately resistant to E.coli in the botanical garden soil. Poultry farm soil sample has the highly resistant to E.coli. (Hassan *et al.,* 2018) [9]

From the sensitivity test with *S.aureus,* ciprofloxacin has the highest zone of inhibition followed by amoxicillin and erythromycin 10 diameters, 32mm, 30 mm, and 22mm respectively. According to (Table IV) the *E.coli* is susceptible to ciprofloxacin and amoxicillin and intermediate to erythromycin. (Valica*et al*., 2017)

Isolated soil bacteria which shows antibiotic activity under normal growth condition by using soil dilution technique. Two fungal culture having zone of inhibition were picked and streaked on separate PDAplates to get pure cultures. In this present study,*E.coli* cuture having the zone of inhibition were picked and streaked on separate Mackankey agar plates to get pure cultures.(Soniasethi*et al.,* 2013)

The poultry of drinking water sources and the presence of antibiotics resistant bacteria increase the risk to human health. It is important to have detailed information regarding such issues. In the present study the different area soil sample to isolate the presence of *E.coli* was resistant to some antibiotics to study the multi-drug resistant.

Antibiotic resistance is difficult to remove even if the release of antibiotic resistance determinants in the environment is discontinued. (Martinez., 2009). In this study 49.50% of the E.coli isolated was resistant to atleast one of the tested antibiotic and 24.0% exhibited multiples resistance are comparable to that in other environment. In this previous study, the overall resistance of e.coli to antimicrobial was high. The result is consistent with the findings of previous studies. In this present study High level of resistance in E.Coli was amoxicillin, and ciprofloxacin. (Iqbal M.K *et al.,* 2002). The relationship between certain soil quality.variable and antibiotic resistance of bacteria or have been reported by several studies, in this present study, we analysed the soil sample and isolate the E.coli. The resistant to different antibiotics had different associations with nutrient concentration. (Maal-bared *et al*., 2013). (Staley *et al*., 2015).

In all agricultural samples, E.Coli showed high resistance rate of ≥ 80% to erythromycin and amoxicillin and ≥60% to Tetracycline. In this study were higher compared to susceptibility patterns reported from previous studies. (Briscoe *et al.,* 2005).

The antibiotic resistant of the present study revealed that the isolated escherichiacoli., showed resistant to multi drugs. These results were line with the reports of (Mohamed *et al.,* 2012), were the resistance of ampicillin (84%), Cefotaxime (77%), Ceftizoxime (55%), amikacin and ofloxacin(25%) and Tetracycline (17%). The reports were higher to the reports of (Firaol *et al*., 2013) to the pencillin G (66.67%), lower to chloromphenical (88.9%), and gentamycin (100%). From to orevious and established antibiotics compared to the newer developed antibiotics. Appearance of resistance against particular antibiotic in a specific region may be due to its frequent and long term use (Kumar *et al.,* 2010).

The antibiotics resistant of the present study revealed that the isolated Escherichia coli, showed resistant to multi drugs. These results were in line with the reports of (Mohamed *et al*.,2012),were the resistance of ampicillin(74%),penicillin (98%) erythromycin (98%), Nalidixic acid (94%), Cephalexin(86%), Amoxycillin (84%), Ciprofloxacin(64%), Tetracycline(54%), cefixime (36%), Gentamycin (54%). The reports were higher to the penicillin (98%), lower to Chloromphenical (88.8%) and Gentamycin. From to be resistant to previous and established antibiotics. Appearance of resistance against a particular antibiotic In a specific region may be due to its frequent and lond term use.

Some natural products like bacteriocin may be used as an alternative type of antibiotic (Kaur*et al.,* 2012). These natural agent which may be inhibit growth of huge number of microbes. *Escherichia coli* were isolated and characterized from the different area soil samples.

Conclusion

Antibiotic is one of the most important commercially exploited secondary metabolites produced by bacteria, fungi, and *Streptomyces* and employed in a wide range. Most of the antibiotics used today are from the microbes. Antibiotic resistance in E.coli is of particular concentrate because it is the most common pathogen in humans. Bacteria are easy to isolate, culture, maintain and to improve their *E.coli* species being the predominant soil bacteria was selected for this study. Multiple antibiotic resistances may be acquired through mobile genetic elements. Antimicrobial resistance has been increased as an emerging worldwide problem in human and veterinary medicine, both in developed and developing countries. Therefore, cautions are necessary to decrease the incidence of multidrug resistant strains of *E. coli* in animals and people. In order to achieve this, good hygienic practices are necessary from the farm to the family table especially in the abattoirs to prevent contamination of cattle and poultry products and abattoir environment with intestinal content. Further study is to be planned to sequence multi drug resistance pattern of *E.coli.*

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