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Isolation and Identification of non-plasmid Multidrug Resistant *E.coli* from Poultry Wastes in Chittagong Region, Bangladesh

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Abstract

In two branches of poultry culture; small local ones and big industrial ones, tetracycline is a common antibiotic, which has been taken as a standard antibiotic in this study. 20 isolates were taken from big poultry farms like Agha Ltd and Denm Poultry. 10 isolates were taken from small local poultry farms like Rahat Poultry and Star Poultry. After collection of samples, total numbers of bacteria with and without tetracycline were counted. In both cases numerous bacterial growths were observed. The normal dose of tetracycline is 30 µg/ml which failed extremely to regulate high bacterial growth. Two dilutions (10^{-3} and 10^{-4}) of sample 1, 2, 3 and 4 were taken and allowed to grow at different concentrations of tetracycline like 30,60 and 100 µg/ml, where bacterial growth was observed. High concentration of antibiotics for example, above 100 µg/ml may be harmful to humans and animals. After performing sensitivity test against other commonly used antibiotics in poultry, it was found that isolated tetracycline-resistant *E. coli* were 100% resistant to penicillin and erythromycin,100 sensitive to imipenem, 93.34% resistant to tetracycline, 23.03% resistant to gentamycin and 53.33% resistant to chloramphenicol. These indicated the multidrug resistant property of isolates. Subsequent agarose gel electrophoresis showed no plasmid DNA band in the gel indicating non-existence of any bacterial plasmid and also proved that the observed resistance was chromosomal gene-mediated or at least not plasmid mediated.

Keywords: *E. coli*; Non-plasmid; Multi-drugs resistant; Poultry wastes

Introduction

The hope ushered by the discovery of antimicrobials has been tainted by the emergence of bacterial strains which are able to resist this therapeutics. Due to the use and misuse of antimicrobials in the last few decades, today's clinically important bacteria are not only single drug resistant but also multiple antibiotics resistant. These multidrug resistant bacteria are increasing public health hazard all over the world [1]. Antimicrobial susceptible bacteria are substantially less responsible for causing infections compared to the antimicrobial-resistant bacteria which actually cause infections leading to higher rates of morbidity and mortality [2]. The reason behind this high rate is that, these antimicrobial-resistant microorganisms are resistant to conventional treatment and can cause serious infection resulting in prolonged illness and greater death risk. Annually, about 440,000 new cases of Multidrug-resistant Tuberculosis (MDR-TB) are reported, causing no less than 150,000 deaths. In most malaria-endemic countries, widespread resistance to earlier generation antimalarial medicines, such as, chloroquine and sulfadoxine-pyrimethamine is seen [3]. Over the past decade, intercontinental spread of methicillin resistant *Staphylococcus aureus* [4] and penicillin resistant *Streptococcus pneumonia* [5], has progressed and has given rise to concerns about increasing resistance of *Salmonella typhi* [6]. It has proved the parochial approach to be a failure. Most antibiotic use is in two areas: in humans in the community, and in animals for growth promotion and prophylaxis. 20-50% human uses of antibiotics are unnecessary and 40-80% agricultural uses of antibiotics are highly questionable [7]. In the Southern Netherlands, almost 80 percent of raw chicken supplied by the grocery stores was found to be containing multidrug-resistant bacteria. When these germs were compared with the speciments collected from hospital patients, researchers found that, the predominant resistant genes were identical [8]. Antimicrobial resistance has been recognized by the World Health Organization (WHO) as a global problem that calls for global response. Keeping the problem in view, WHO issued the global principles for the containment of antimicrobial resistance in animal intended for food. After some recommended interventions, the WHO global strategy for the containment of antimicrobial resistance will hopefully enable local authorities to reduce the spread of resistance and slow down its emergence in diverse setting [9,10]. These guidelines recommend prudent use of antimicrobials and the establishment of surveillance programmes for antimicrobial consumption and resistance and further research as well.

Collection of sample

Samples were collected from four poultry farms

1) Agha Poultry Ltd, Roufabad, Hathajari, Chittagong
2) DENM Poultry Farm, North Fatehabad, Chittagong
3) Star Poultry, University of Chittagong campus area
4) Rahat Poultry, Mogoltuli, Chittagong

Sample-1 (Agha Poultry) and Sample-2 (DENM Poultry) are big commercial poultry farms. Sample-3 (Star Poultry) and Sample-4

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(Rahat Poultry) are small local poultry farms. Samples collected from each of these poultries were- a) raw feces from the inside of the farms, b) feces from the open fields beside the farms which were thrown away as waste products.

Transportation of the sample

After collection the samples were placed in a sterile ice-bag containing ice and were transported to the laboratory of Department of Microbiology, University of Chittagong.

Processing of samples

Samples were allowed to reach room temperature and then 10 gm of fresh fecal sample was mixed with 90ml of sterile normal saline and shook to form homogenous mixture. All samples were mixed by vigorous shaking.

Bacteriological count

All the bacteriological enumerations were carried out by pour plate method. In this case total number of bacteria and total number of resistant bacteria were counted [11].

Total Viable Count (TVC) with and without antibiotic

1 ml of from 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions were poured into different sterile Petri plates. The Nutrient agar media (temperature 45°C) were poured into each petri-plate. After solidification, the plates were incubated at 37°C for 24 hours at inverted position. After 24 hours, plates with 30-300 bacterial colonies were counted.

There is a difference between total viable count without antibiotic and total viable count with antibiotic. In case of total viable count with antibiotic, antibiotics (30 µg/ml tetracycline) were mixed to the sterilized media (temperature 45°C) and were shaken well before plating.

Transferring single colonies from NA plates to EMB agar media

Single colonies were picked up randomly by sterile tooth picks from plates (with different concentration of Tetracycline). The colonies were then streaked on individual EMB agar containing 30 µg/ml tetracycline. The EMB plates were incubated at 37° C for 24 hours.

Transferring to broth culture

After incubation, presence of growth with green metallic sheen was observed on the EMB plates. One loopful from such growths was transferred randomly to 3 ml of nutrient broth (in 10ml screw cap tubes) containing 30 µg/ml tetracycline samples. 30 such growths (10 from sample-1, 10 from sample-2, 5 from sample-3 and 5 from sample-4) were transferred patching from all of the samples. The 30 culture tubes were then allowed for incubation at 37°C for 24 hours with loose capping and vigorous shaking of over 250 rpm.

Identification of the Isolated *E. coli*

Microscopic examination of morphology bacteria

The size, shape, arrangements and Gram reactions of the 24 hour bacterial cultures were observed in a microscopical field [12].

Conventional biochemical test for the identification of *E. coli*

Conventional Biochemical tests were carried out for the identification of *E. coli*. The tests are- Indole test, Methyl-red test, Voges-proskauer test, Citrate test and Motility test. Tetracycline (30 µg/ml) was present in all biochemical tests.

Antimicrobial susceptibility of the microorganisms to antibiotics

The standard disc diffusion method also known as Kirby Bauer method [13] was used for the in vitro determination of the sensitivity to the antimicrobial agents.

Antibiotic disc used

Antibiotics were chosen so that some of them were used during sample collection (e.g. tetracycline), some of them were continuously used in the poultry in addition to the running antibiotics, some of them were moderately or rarely used in poultry farms, some of them were not used (e.g. Imipenem and Gentamycin) (Table 1).

Plate preparation

A cotton swab was dipped in the suspension prepared in compliance with McFarland solution, excess fluid was removed by pressing and rotating the cotton bar inside the wall of the tube just above the fluid level. Then the swab was streaked over the surface of the Muller-Hinton agar medium to obtain uniform inoculums and some plates were also prepared by pour plate method.

Preparation and application of the disc to the plates

The discs were then placed on the surface of the seeded plates at appropriate spatial arrangement by using a sterile forceps. Then the plates were inoculated at 37°C for 24 hours and observed for the clear zone of inhibition.

Observation of clear zone of inhibition

After incubation the zones of complete inhibition were measured by using MD8 Scan Zone Reader.

Plasmid isolation

Plasmid extraction procedure was carried out following the protocol developed by ICDDRB. The extracted plasmid was then isolated using a horizontal 1% Agarose Gel Electrophoresis technique.

Preparation of the sample

The pure cultures were transferred to 10 ml screw cap tubes containing 3 ml Luria Bertani (LB) broth with 30 µg/ml tetracycline. The broths were then incubated at 37°C with loose capping and vigorous shaking (200 rpm) for overnight. Then inoculums were transferred to another 3 ml LB broth at a 1:200 ml rate containing same concentration of tetracycline and incubated for 4-6 hours at 37°C with loose capping and vigorous shaking (200 rpm). After sufficient growth

Antibiotics name	Symbol	Concentration of antibiotics applied
Tetracycline	T	30 µg
Gentamycin	G	10 µg
Imipenem	I`	10µg
Chloramphenicol	C	30 µg
Penicillin	P	10 µg
Erythromycin	E	15 µg

Table 1: Six antibiotics that were tested against the *E. coli* isolates using standard disc.

with slight turbidity the incubation stopped and the cells were prepared for extraction.

Plasmid extraction

1.0ml of overnight culture was taken in an Eppendorf's tube (1.5ml) and cells were collected by centrifugation for 7 minutes at 12,000 rpm. The supernatant was discarded and the pellet was thoroughly suspended in 100 µl of solution I and the solution was kept at room temperature (32°C) for 10 min.

Then 200 µl of solution II (lysis solution) was added and mixed gently by inverting the tube for a few times. After that 150 µl of ice-cold solution III (neutralizing solution) was added and mixed vigorously by vortexing for a few seconds. The tubes were kept on ice for 5 minutes. The mixture was then centrifuged at 12,000 rpm for 15 minutes to pellet the chromosomal DNA. The clear supernatant (approximately 400 µl) was taken to fresh Eppendorf's tubes. Then two volumes of cold 95% ethanol (800 µl) were added in each tube and vortexes for a few seconds to mix well. It was then kept in room temperature for about 20 minutes for DNA precipitation. The precipitated DNA was collected by centrifugation for 15 minutes at 12,000 rpm. The supernatant was discarded and the pellet was dried in a drier at 45°C for 20 minutes. Finally the dried DNA was dissolved in 30 µl TE buffer and kept at 4°C.

Separation of plasmid DNA by agarose gel electrophoresis

Plasmid DNA was separated by horizontal electrophoresis in 1% agarose slab gels in a Tris-Acetate EDTA (TAE) buffer at room temperature at 80 volt (50 mA) for 3 h. briefly, 30 µl of plasmid DNA solution was mixed with 3 µl of tracking dye (Appendix) and was loaded into the individual well of the gel. The gel (5mm thick) was stained with 0.5 µg/ml of ethydium bromide for 15 min at room temperature and then distained with distilled water for 10 min.

Results

Bacterial enumerations

Total count of bacteria with and without antibiotics (tetracycline): Total number of bacteria (without antibiotics) in the samples collected from Agha Ltd, Denm poultry (big commercial poutries) and Rahat poultry, Star poultry (small local poultries) were counted and the results were given in Table 2 and presented in the Figure 1. The numbers of total bacteria differ from sample to sample. Total average count of the fecal wastes collected from a small local poultry -Rahat poultry showed highest number of bacteria 34510000/ml (sample-4). The second highest count (31140000/ml) was also from a small local poultry -Star poultry (sample-3). Total average count of sample- 1(big commercial poultry-Agha Ltd.) and sample-2 (big commercial poultry-Demn poultry) were 11276667/ml and 15970000/ml respectively. The highest count (from small local poutry Rahat poultry) was 3.07 times greater than that of lowest count (from a big commercial poultry-Agha Poultry). In total bacterial count with antibiotics (tetracycline) of same sample (sample-4, Star poultry, small local poultry) showed

Sample	Dilution	No.of Colony	No.of microrganisms/ ml	Average
Sample – 1 AGHA	10^{-2} 10^{-4} 10^{-6}	Too Numerous 83 33	× 830000 33000000	11276666.67
Sample – 2 DEMN	10^{-2} 10^{-4} 10^{-6}	Too Numerous 91 47	× 910000 47000000	15970000
Sample – 3 STAR	10^{-2} 10^{-4} 10^{-6}	Too Numerous 142 92	× 1420000 92000000	31140000
Sample – 4 RAHAT	10^{-2} 10^{-4} 10^{-6}	Too Numerous 153 102	× 1530000 102000000	34510000

Table 2: Total Count of Bacteria without Antibiotics (tetracycline).

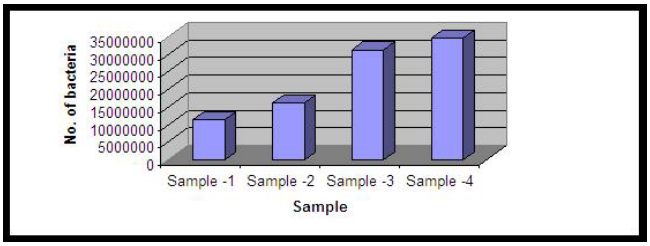

Figure 1: Result of total viable count of four types of samples collected from poulties.

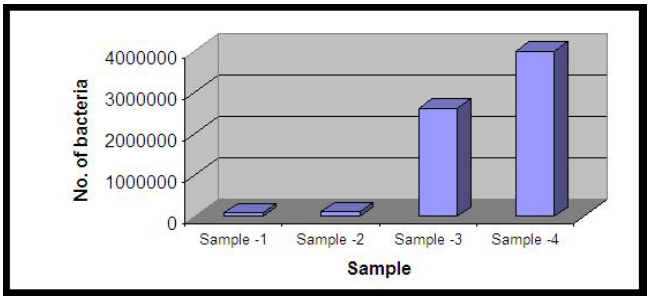

Figure 2: Total resistant bacterial count in the samples 1,2,3,4.

Sample	Dilution	No.of Colony	No.of microorganisms/ ml	Average
Sample – 1	10^{-2} 10^{-4} 10^{-6}	100 23 0	10000 230000 ×	80000
Sample – 2	10^{-2} 10^{-4} 10^{-6}	120 33 0	12000 330000 ×	114000
Sample – 3	10^{-2} 10^{-4} 10^{-6}	Too Numerous 83 7	× 830000 7000000	2610000
Sample – 4	10^{-2} 10^{-4} 10^{-6}	Too Numerous 94 11	× 940000 11000000	3980000

Table 3: Total Count of Bacteria with Antibiotics (tetracycline).

Sample	Dilution	Concentration of tetracycline (µ/ml)	No. of Colony	No. of Bacteria/ml
1	10^{-3} 10^{-3} 10^{-3}	30 60 100	53 24 13	53×10^{-3} 24×10^{-3} 13×10^{-3}
2	10^{-3} 10^{-3} 10^{-3}	30 60 100	77 39 19	77×10^{-3} 39×10^{-3} 19×10^{-3}
3	10^{-3} 10^{-3} 10^{-3}	30 60 100	103 61 27	103×10^{-3} 61×10^{-3} 27×10^{-3}
4	10^{-3} 10^{-3} 10^{-3}	30 60 100	185 73 53	185×10^{-3} 73×10^{-3} 53×10^{-3}

Table 4: Bacterial count (dilution 10-4) with different concentration of tetracycline.

Sample	Dilution	Concentration of tetracycline (μ/ml)	No. of Colony	No. of Bacteria/ml
1	10^{-4} 10^{-4} 10^{-4}	30 60 100	33 11 3	33×10^{-4} 11×10^{-4} 3×10^{-4}
2	10^{-4} 10^{-4} 10^{-4}	30 60 100	53 24 6	53×10^{-4} 24×10^{-4} 6×10^{-4}
3	10^{-4} 10^{-4} 10^{-4}	30 60 100	91 33 11	91×10^{-4} 33×10^{-4} 11×10^{-4}
4	10^{-4} 10^{-4} 10^{-4}	30 60 100	30 60 100	102×10^{-4} 43×10^{-4} 13×10^{-4}

Table 5: Bacterial count (dilution 10^{-4}) with different concentration of tetracycline.

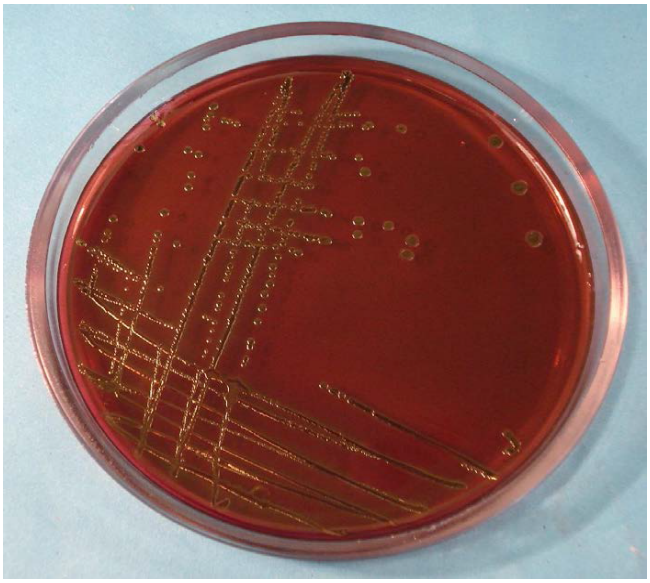

Figure 3: Tetracycline (30 μl/ml) Resistant *E. coli* on EMB.

highest bacterial count (3980000) and sample-1 (Agha, Big commercial poultry farm) exhibited the lowest bacterial count (8000/ml). The highest one was 497.5 times greater than lowest one. It is important to note that the amount of tetracycline resistant bacteria in local poultries (sample-1 and 2) is much higher than that of sample 3 and 4 (Figure 2 and Table 3-5).

Isolation and identification of tetracycline resistant *E. coli*

A total of 30 individual colonies of *E. coli* were isolated and were characterized according to the biochemical properties. Following figures show the characteristic metallic sheen on EMB agar plate of the isolates and the biochemical properties (Figures 3-7).

Antimicrobial Susceptibility

Antimicrobial susceptibility patterns of the isolates

Six antibiotics were tested against the *E. coli* isolates using standard disc.

1. Tetracycline (T,30 μg)
2. Gentamycin (G,10 μg)
3. Imipenem (I,10 μg)
4. Chloramphenicol (C,30 μg)
5. Penicillin (P,10 μg)
6. Erythromycin (E,15 μg)

After performing sensitivity test it was found that isolated tetracycline-resistant *E. coli* were 100% resistant to penicillin and erythromycin, 100% sensitive to imipenem, 93.34% resistant to

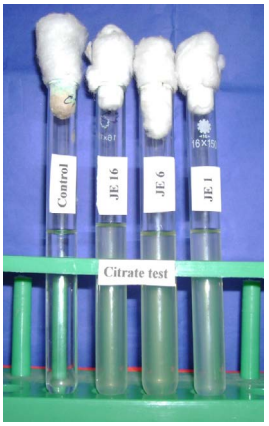

Figure 4: Citrate Test.

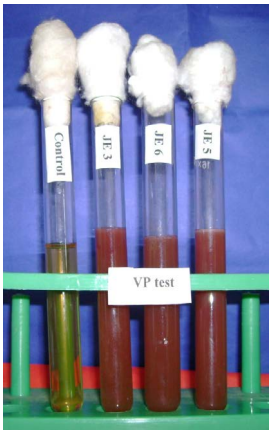

Figure 5: VP Test.

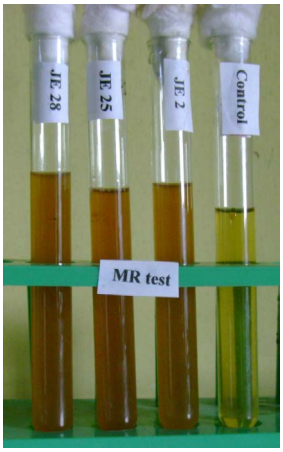

Figure 6: MR Test.

Figure 7: Motility Test.

Antimicrobial agents (µg)	Diffusion zone breakpoints (mm)
Aminoglycosides	
Gentamycin	≤ 12
Cephalosporins	
Penicillin	≤ 13
Imipenem	≤ 13
Phenicols	
Chloramphenicol	≤ 12
Macrolides	
Erythromycin	≤ 14
Tetracycline	
Tetracycline	≤ 14

Table 6: Standard range of antimicrobial susceptibility.

tetracycline, 23.03% resistant to gentamycin and 53.33% resistant to chloramphenicol (Figure 8 and Tables 6-8).

Total 30 isolates were subjected to plasmid DNA extraction and they were analyzed in 1% Agarose. The results are negative and no band was found (Figure 9).

Discussion

Random use of antibiotics without medical indication in Poultry and adult dairy cows are a common phenomenon these days. This contributes to the increase of antimicrobial resistance and indirectly exposes human beings to these pathogens [14]. In this study, poultry, a popular and widespread business was selected to observe its contribution to the development of multi-drug resistant *E. coli*. We have divided Poultry two branches-small local culture and big industrial culture. Various types of antibiotics are being used in these poultry industry. The most common types of antibiotic that is used in poultry are tetracycline-which was used as standard antibiotic in this study. Other most common type of antibiotics like penicillin, imepenem, chloramphenicol, erythromycin and gentamycin were used to observe multi-drug resistance. 20 isolates were taken from big poultry farms like Agha Ltd and Denm Poultry. 10 isolates were taken from small local poultry farms like Rahat Poultry and Star Poultry. After collection of sample, total number of bacteria with and without antibiotics was counted. In both cases numerous bacterial growths were observed. The normal dose of tetracycline is 30 µg/ml which failed extremely to regulate high bacterial growth. The samples labeled with number 1, 2, 3, and 4 were allowed to grow at different concentrations of tetracycline (30, 60 and 100 µg /ml) where bacterial growth was observed. After performing sensitivity test against other commonly used antibiotics in poultry, it was found that isolated tetracycline-resistant *E. coli* were 100% resistant to penicillin and erythromycin, 100% sensitive to imipenem, 93.34% resistant to tetracycline, 23.03% resistant to gentamycin and 53.33% resistant to chloramphenicol. These indicated the multidrug resistant property of isolates. A statistically significant [12] Increase in antibiotic resistance was observed among outpatient and inpatient isolates of *E. coli*. Subsequent Agarose Gel Electrophoresis showed no plasmid-DNA band in the gel indicating non-existence of any bacterial plasmid proving that observed resistance was chromosomal gene-mediated or at least not plasmid mediated. Observation of the multi-drug resistance character of poultry fecal isolates is a terrible warning to natural environment [15,16]. The poultry feces used by farmers as manure can poison the crop. Poultry feces is also used as a common feed for fish, so these fish containing multi-drug resistant culture of bacteria like *E. coli* can be deadly for humans and animals, that is, for any fish eaters. Antibiotics resistance in bacteria associate with food animals and the use of antibiotics for agricultural purposes, particularly for growth enhancement, contributed to the increased prevalence of antibiotic-resistant bacteria. Our finding proposed that proper antibiotics should be used at proper doses to avoid the development of multi-drug resistant bacteria. To perform these, skilled workers

Antimicrobial agents	Resistant (R) isolates (%)	Intermediate (I) isolates (%)	Sensitive (S) isolates (%)
Gentamycin	7 (23.3%)	0	23 (76.7%)
Penicillin	30 (100%)	0	0
Imipenem	0	0	100 (100%)
Chloramphenicol	16 (53.33%)	0	14 (46.67%)
Tetracycline	28 (93.24%)	1 (3.34%)	1 (3.34%)
Erythromycin	30 (100%)	0	0

Table 7: Susceptibilities of 30 isolates from sample 1, 2, 3 and 4 to different antibiotics.

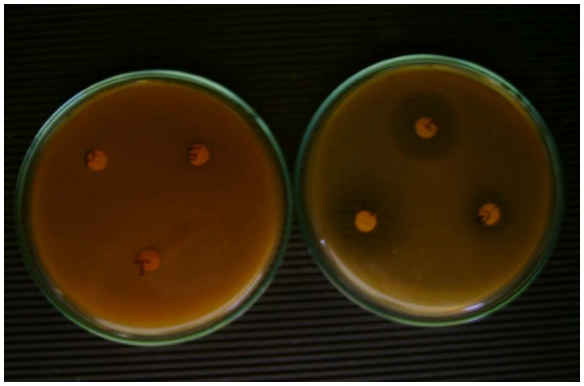

Figure 8: Antibiotic sensitivity test.

	Antibiotics	Concentration (µg/ml)	Zone of inhibition (mm)	Remarks			Antibiotics	Concentration (µg/ml)	Zone of inhibition (mm)	Remarks
JE1	Penicillin	10µg	2	R		JE16	Penicillin	10µg	0	R
	Gentamycin	10 µg	14	R			Gentamycin	10 µg	8	R
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	2	R
	Tetracycline	30 µg	0	R			Tetracycline	30 µg	16	I
	Chloramphenicol	30 µg	0	R			Chloramphenicol	30 µg	0	R
	Imipenem	10 µg	29	S			Imipenem	10 µg	22	S
JE2	Penicillin	10µg	0	R		JE17	Penicillin	10µg	0	R
	Gentamycin	10 µg	18	R			Gentamycin	10 µg	24	S
	Erythromycin	15 µg	9	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	10	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	20	S			Chloramphenicol	30 µg	3	R
	Imipenem	10 µg	41	S			Imipenem	10 µg	36	S
JE3	Penicillin	10µg	0	R		JE18	Penicillin	10µg	0	R
	Gentamycin	10 µg	15	R			Gentamycin	10 µg	27	S
	Erythromycin	15 µg	6	R			Erythromycin	15 µg	6	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	11	R
	Chloramphenicol	30 µg	0	R			Chloramphenicol	30 µg	9	R
	Imipenem	10 µg	32	S			Imipenem	10 µg	29	S
JE4	Penicillin	10µg	0	R		JE19	Penicillin	10µg	0	R
	Gentamycin	10 µg	4	R			Gentamycin	10 µg	19	S
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	3	R
	Tetracycline	30 µg	10	R			Tetracycline	30 µg	9	R
	Chloramphenicol	30 µg	9	R			Chloramphenicol	30 µg	0	R
	Imipenem	10 µg	28	S			Imipenem	10 µg	27	S
JE5	Penicillin	10µg	5	R		JE20	Penicillin	10µg	5	R
	Gentamycin	10 µg	9	R			Gentamycin	10 µg	20	S
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	13	R
	Chloramphenicol	30 µg	0	R			Chloramphenicol	30 µg	19	S
	Imipenem	10 µg	33	S			Imipenem	10 µg	41	S
JE6	Penicillin	10µg	0	R		JE21	Penicillin	10µg	0	R
	Gentamycin	10 µg	19	S			Gentamycin	10 µg	16	S
	Erythromycin	15 µg	5	R			Erythromycin	15 µg	9	R
	Tetracycline	30 µg	9	R			Tetracycline	30 µg	6	R
	Chloramphenicol	30 µg	19	S			Chloramphenicol	30 µg	0	R
	Imipenem	10 µg	37	S			Imipenem	10 µg	24	S
JE&	Penicillin	10µg	4	R		JE22	Penicillin	10µg	0	R
	Gentamycin	10 µg	23	S			Gentamycin	10 µg	24	S
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	3	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	22	S
	Chloramphenicol	30 µg	18	S			Chloramphenicol	30 µg	19	S
	Imipenem	10 µg	42	S			Imipenem	10 µg	24	S
JE8	Penicillin	10µg	9	R		JE23	Penicillin	10µg	0	R
	Gentamycin	10 µg	16	S			Gentamycin	10 µg	23	S
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	9	R
	Tetracycline	30 µg	13	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	11	R			Chloramphenicol	30 µg	9	R
	Imipenem	10 µg	31	S			Imipenem	10 µg	26	S
JE9	Penicillin	10µg	0	R		JE24	Penicillin	10µg	0	R
	Gentamycin	10 µg	16	S			Gentamycin	10 µg	24	S
	Erythromycin	15 µg	2	R			Erythromycin	15 µg	11	R
	Tetracycline	30 µg	2	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	20	S			Chloramphenicol	30 µg	25	S
	Imipenem	10 µg	26	S			Imipenem	10 µg	22	S

JE10	Penicillin	10µg	0	R		JE25	Penicillin	10µg	0	R
	Gentamycin	10 µg	20	S			Gentamycin	10 µg	19	S
	Erythromycin	15 µg	7	R			Erythromycin	15 µg	8	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	19	S			Chloramphenicol	30 µg	22	S
	Imipenem	10 µg	31	S			Imipenem	10 µg	27	S
JE11	Penicillin	10µg	0	R		JE26	Penicillin	10µg	0	R
	Gentamycin	10 µg	21	S			Gentamycin	10 µg	17	S
	Erythromycin	15 µg	0	R			Erythromycin	15 µg	8	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	0	R			Chloramphenicol	30 µg	25	S
	Imipenem	10 µg	34	S			Imipenem	10 µg	23	S
JE12	Penicillin	10µg	0	R		JE27	Penicillin	10µg	0	R
	Gentamycin	10 µg	26	S			Gentamycin	10 µg	21	S
	Erythromycin	15 µg	4	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	11	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	25	S			Chloramphenicol	30 µg	0	R
	Imipenem	10 µg	44	S			Imipenem	10 µg	26	S
JE13	Penicillin	10µg	0	R		JE28	Penicillin	10µg	0	R
	Gentamycin	10 µg	22	S			Gentamycin	10 µg	24	S
	Erythromycin	15 µg	3	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	15	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	0	R			Chloramphenicol	30 µg	0	R
	Imipenem	10 µg	37	S			Imipenem	10 µg	34	S
JE14	Penicillin	10µg	0	R		JE29	Penicillin	10µg	0	R
	Gentamycin	10 µg	22	S			Gentamycin	10 µg	24	S
	Erythromycin	15 µg	2	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	9	R			Tetracycline	30 µg	0	R
	Chloramphenicol	30 µg	19	S			Chloramphenicol	30 µg	4	S
	Imipenem	10 µg	36	S			Imipenem	10 µg	21	S
JE15	Penicillin	10µg	0	R		JE30	Penicillin	10µg	0	R
	Gentamycin	10 µg	8	R			Gentamycin	10 µg	17	S
	Erythromycin	15 µg	8	R			Erythromycin	15 µg	0	R
	Tetracycline	30 µg	15	R			Tetracycline	30 µg	11	R
	Chloramphenicol	30 µg	8	R			Chloramphenicol	30 µg	22	S
	Imipenem	10 µg	33	S			Imipenem	10 µg	24	S

Table 8: Antimicrobial susceptibility of all of the poultry isolates showing different zone of inhibition (mm).

with sound knowledge of antibiotics are essential. For personal-small poultry farm, the related individuals should take training on the use antibiotics. The waste of poultry should be disposed off properly to avoid the spread of multi-drug resistant bacteria in the environment.

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