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Isolation and characterization of Oxytetracycline resistance *E. coli* in Al Muthanna Veterinary hospital using Multiplex PCR

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Abstract

Different antibiotics have used in cure and control of infections in veterinary practice, therefore, the non-prudent use can lead to the development of antibiotic resistant microorganisms. Therefore, this study was designed to Identify the presence of drug-resistant bacteria and to investigate the prevalence of oxytetracycline resistance gene tet (A) in the isolated and in vitro antibiotic resistance *E. Coli.* A total of 53 clinical cases (37 sheep, 6 goats, 9 cow, and one cat) were presented to Al-Muthanna veterinary hospital with different clinical signs. A sterile samples were collected from these cases and cultured in different culture media. Consequently, Gram stain and biochemical tests were done for identification. The total number of isolated bacteria were 53 isolates. Ten out of 53 bacterial isolates were *E. coli* and were resistance for tetracycline and oxytetracycline. These isolates were tested to determine the prevalence of tetracycline resistance genes tet (A) using multiplex PCR. Nine out of ten *E. Coli* isolates (9/10, 90 %) were carried tetA gene. PCR amplified the tet (A) gene was done with two sets of primers targeting the tetracycline efflux gene (tetA). In conclusion, antimicrobial resistant (AMR) bacteria were identified in this study. Moreover, tetA resistance gene found in 90% of the tested resistance *E. coli*. The Authors recommend doing another future investigation that includes the high number of bacterial isolates and determines other antimicrobial genes responsible for transferring the resistance between different kinds of bacteria. Besides, antimicrobial drugs should be used wisely and prohibited providing antimicrobial products without a prescription.

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Keywords: Al-Muthanna governorate, drug-resistant bacteria, *E. coli*, tetracycline efflux gene (tetA), multiplex PCR

Introduction

With amplified antibiotics usage over current decades, there has been an emerging alarm about the speeded development of antibiotic-resistant bacteria in the environment. The spread of antibiotic-resistant bacteria in the environment is

depending on the presence and transfer of resistance genes among microorganisms, and selection pressure to keep these genes in a population (Cizman, 2003). Antimicrobials resistance appears from the use of antimicrobials in animals, and subsequent transfer of resistance genes and bacteria among animals and animal products and the environment. The development of resistance has been slow in some countries by restriction of antimicrobial use in feed and treatment of the diseased animal. Moreover, improved management practices, wider use of vaccines, in addition to the introduction of probiotics as alternatives to growth-promoting and prophylactic applications of antimicrobials in agriculture (Cizman 2003; Aarestrup. 2004; Li *et al.,* 2010). *E. coli* is commonly recognized in human and animal intestinal tracts, and any fecal contamination during slaughtering of the animal leads to transfer of this microorganism to consumers. *E. coli* are determined as important pathogens of Colibacillosis in animals, and can also cause severe human diseases such as hemorrhagic colitis and hemolytic uremic syndrome (Riley *et al.,* 1983; Chansiripornchai, 2009; Ferens and Hovde, 2011). The treatment of the diseases caused by this bacterium commonly needs antimicrobial therapy. The decision to use antimicrobial treatment depends on the susceptibility of the microorganism and the pharmacokinetics of the drug for achieving the requested therapeutic concentration at the site of infection and then clinical efficacy (McKellar *et al.,* 2004). Meanwhile, veterinary practitioners have a limited choice of antimicrobials due to antimicrobial resistance issues and human health concerns. Moreover, the repeated and improper use of antibiotics has led to an increasing rate of antimicrobial resistance (Mooljuntee *et al.,* 2010). Tetracyclines are antibiotics that act to inhibit bacterial growth by interfering with protein synthesis. The emergence of bacterial resistance to these antibiotics has nowadays limited their use. Three different mechanisms of tetracycline resistance have been identified so far: tetracycline efflux, ribosome protection, and tetracycline modification (Arzese *et al.,* 2000; Blake *et al.,* 2003). The more common resistance mechanism in Gram-negative bacteria is the energy-dependent efflux pump system, which is encoded by tetA, tetB, tetC, tetD, and tetG genes.

In Iraq, antimicrobials are freely available and used for human and animal without prescription from the specialist and estimates of its use vary widely. This misuse has led to an increase in the number of resistant bacteria to multiple antimicrobial agents involved in clinical infections. This situation is threatening the effectiveness of even the most reliable antibiotics used to treat bacterial infections.

Due to the excessive use of tetracycline derivatives in veterinary practice in Iraq, for more than three decades in the treatment of diseased animals. The veterinary daily clinical observations revealed that tetracycline is routinely misused and abused, moreover its efficacy was decreased in the treatment of acute and chronic clinical cases. Nowadays, Veterinarians in practice are searching for another and new antimicrobial products. Recently, molecular techniques, especially polymerase chain reaction (PCR), have been widely used to study antimicrobial resistance genes. Review of literature revealed a lack of information on the molecular studies regarding tetracycline resistance bacteria in Iraq. Therefore, this study was designed to: identify the presence of drug-resistant bacteria and investigate the prevalence of tetracycline resistance genes tet (A) in the *E. coli* isolates that are resistance in vitro to antibiotics.

Materials and Methods

Sample collection and preliminary bacterial culture

In this study, a total of 53 different clinical samples were collected from cow, sheep, goat, and cats, which presented to Al-Muthanna veterinary hospital from October to December 2017. The samples were collected aseptically using sterile swab after disinfected the area of the collection with 70% alcohol to minimize surface contamination. All samples were transferred in cool box to the clinical pathology laboratory/ College of Veterinary Medicine/ Al-Muthanna University. All samples were culture on 5% sheep blood and MacConkey agar (Figure. 1), and incubated for 18 to 24 h at 37 °C. All bacterial isolates were re-cultured on differential media and nutrient agar for further identification procedures. Colonies with the typical color and appearance of *E. coli* were picked and streaked again on blood and EMB agars. Later on, all bacterial isolates that showed greenish metallic sheen growth on EMB were suggestive of E. coli, and re-inoculated on nutrient slants for further biochemical tests. The E. coli isolates were stored in tryptic soy broth with 15% glycerol at –20 °C (Mooljuntee *et al.,* 2010).

Figure. 1: Shows the preparation of media

Conventional biochemical test

All Gram-positive and negative bacterial isolates including *E. coli* suspected colonies on EMB were further screened using biochemical tests namely; Simmon citrate, Urea, Tripple Iron Sugar (TSI), sulfate, Indole, Motility (SIM), Methyl Red (MR),

Vogesproskeur (VP), oxidase and catalase. Various reactions of the tests such as color change, motility, and gas formation were used to interpret results as either positive or negative after 24hour incubation. These tests were carried out as described in the methodology of (Carter, 1986) (Figure. 2 & 3, 4).

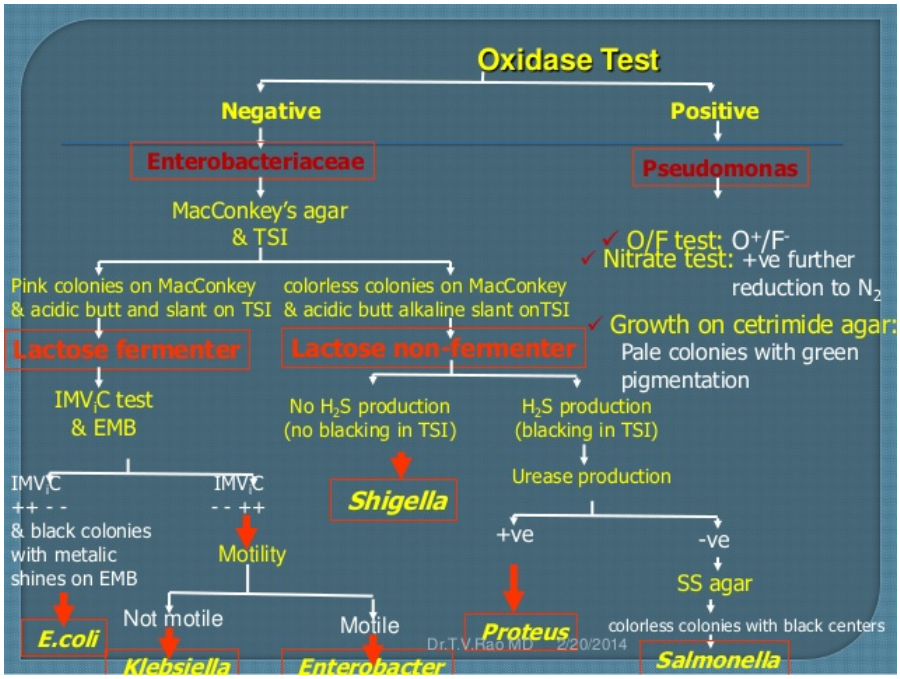

Figure.2: Shows the steps in the identification of Gram positive and negative bacteria

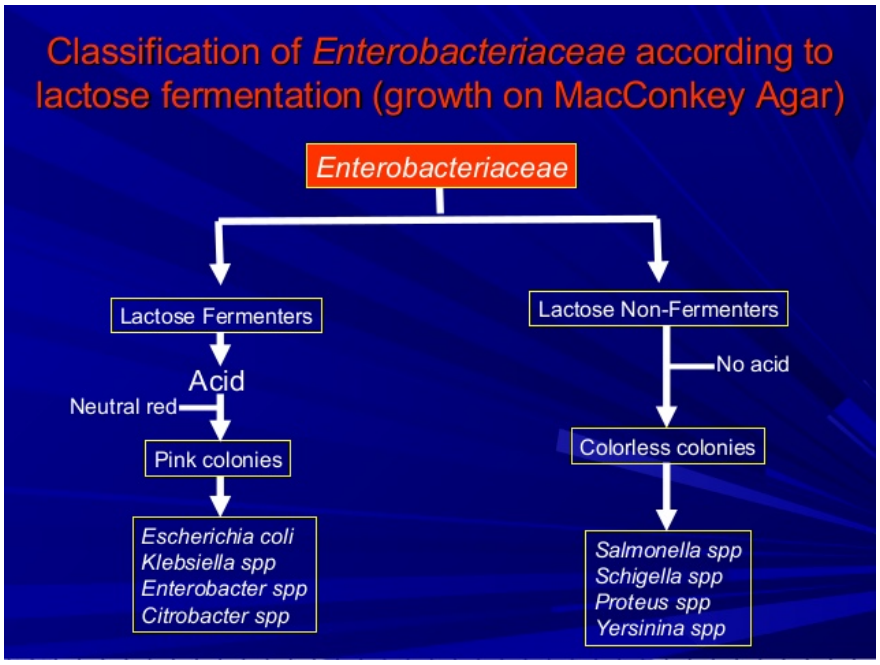

Figure.3: Shows the steps for the identification of *E. coli*

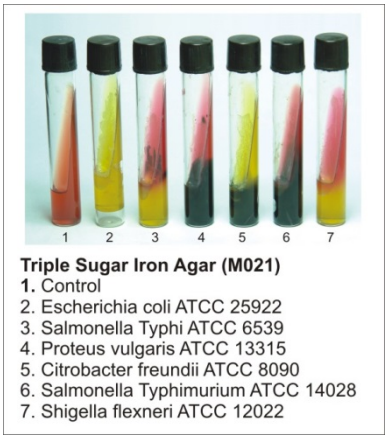

Figure. 4: Shows the stander reading of TSI

Determination of Antibiotic Susceptibility of *E. coli* isolates

Antibiotic susceptibility profile test of each isolate was determined using the disc diffusion method according to the CLSI (Clinical and Laboratory Standards Institute, 2009) protocol. The suspension turbidity was adjusted to match 0.5 McFarland's standard. Ten µl of the suspension was then dispensed and spread on Mueller-Hinton agar plates to create a uniform lawn. The pre-inoculated plates were used for the disc diffusion test. The isolates were tested with a panel of 6 antibiotic discs: tetracycline (T30), and oxytetracycline (TE10), streptomycin (S25), penicillin (P10), trimethoprim-sulphamethoxazole (SXT25) and Chloramphenicol (C10) discs. The antibiotic discs were put on the surface of each of the pre-inoculated Mueller-Hinton plates using a disc dispenser (Oxoid UK) and the plates incubated aerobically at 37 ^{0}C for 24 hours. The diameters of the antibiotic inhibition zones were measured to the nearest millimeter (mm) using a digital meter ruler (Figure. 4) after the incubation period. The isolates were classified as susceptible (S), intermediate resistant (I) or resistant (R) according to the CLSI criteria. Antibiotic discs were obtained from the Oxoid Company (UK) (Figure.5).

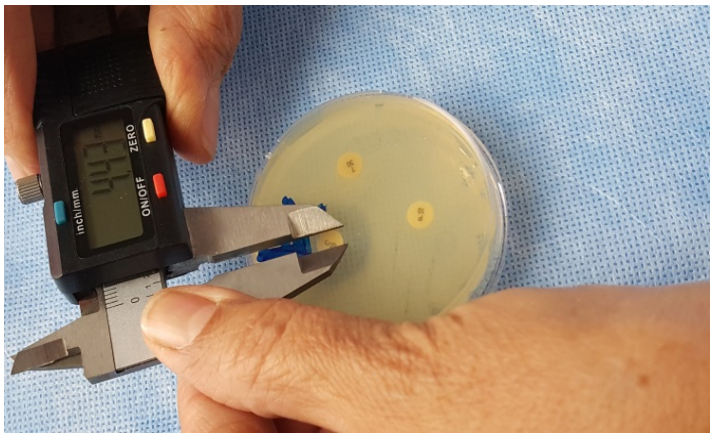

Figure. 5: Shows the digital meter ruler that used for measure the inhibition zones

Detection of Tetracycline (tet) Resistance Genes

According to the instructions of the company (Qiagen/German) the DNA was extracted and purified using overnight bacterial isolates culture and tetAC F, tetAC R, Tet(A)-F and Tet(A)-R primes Table.1). Meanwhile, PCR Amplification cocktail Tet(A) was presented in the table. 2. The PCR Amplification Program for Tet(A) and PCR Amplification Program tetAC were shown in Table. 3 and 4 respectively.

Table. 1: Shows the designed primers use for confirmation of the tet A resistance gene

Prime	**Sequence (5'-3')**	**Size of amplified product (bp)**	**References**
*tet*AC F	5'CGCYTATATYGCCGAYATCAC-3'	417bp	(Balasubramaniam, *et al.,* 2003)
tetAC R	5'CCRAAWKCGGCWAGCGA-3		
Tet(A)-F	5'- GTGAAACCCAACATACCCC-3'	888bp	(Maynard *et al*., 2003)
Tet(A)-R	5'-GAAGGCAAGCAGGATGTAG-3		

Table. 2: PCR Amplification cocktail Tet(A)

Reagents		Quantity
1.	Green Master Mix. Tube	10 µl
2.	F Primer	1 µl
3.	R Primer	1 µl
4.	DNA template	6 µl
5.	Nuclease-free water	32 µl

Table. 3: PCR Amplification Program Tet(A)

Stages		Steps	Temperature (C˚)	Time	No. of cycles
First		Initial Denaturation	94	5 min	1
Second	I	Denaturation	94	40 s	40
	II	Annealing	57	1	
	III	Extension	72	1 min	
Third		Final Extension	72	10 min	1

Table. 4: PCR Amplification Program tetAC

Stages		Steps	Temperature (˚ C)	Time	No. of cycles
First		Initial Denaturation	94	5 min	1
Second	I	Denaturation	94	45 s	30
	II	Annealing	55	1 min	
	III	Extension	72	45s	
Third		Final Extension	72	10 min	1

Results

Microbiological observations

Fifty-three bacterial isolates were obtained from different animal specimens including 9, 37, 6 and one from a cow, sheep, goat, and cat respectively (Table. 5).

Table. 5: Shows the total number and bacterial isolates from animals

Species	Number of animals	No of isolates
Cow	9	9
sheep	37	37
goat	6	6
cat	1	1
Total	53	53

All isolates revealed typical Gram staining, colonies morphological appearance and biochemical tests (Figure. 6). Ten selected E. coli isolates were identified according to its particular reaction in biochemical assays (Table. 6).

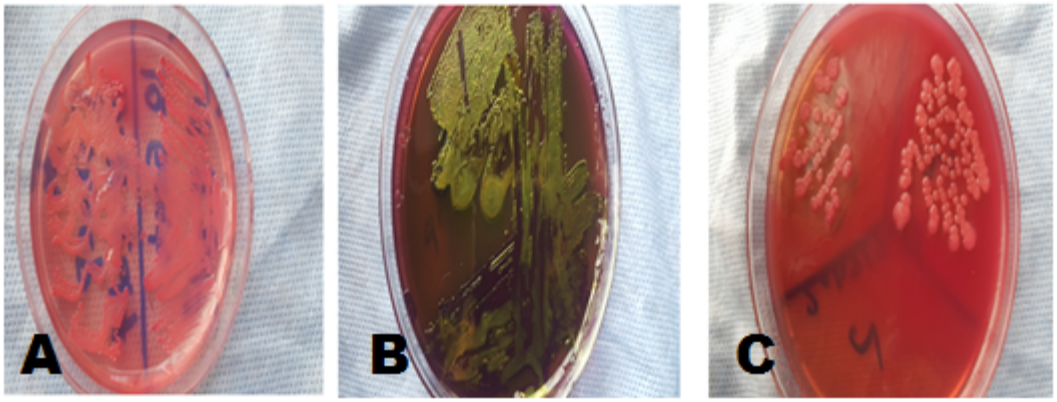

Figure.6: Shows the different bacterial isolates on culture media A. MacConkey agar: B. EMB agar: C. Blood agar.

In samples collected from sheep, the number of bacterial isolates was 25, 6, 3, 1 and 1 for *E. coli*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Klebsiella*, *Proteus*, and *Salmonella* respectively. The most common isolated bacteria was the E. coli. The majorities of these isolates were resistant to the panel of antibiotic discs used in this study. The percentages of resistance were 81.57%, 92.1%, 84.21%, 89.47%,94.7%, 84.21% for S, Te, C, T, P and SXT respectively (Table.7). In samples collected from goat, the number of bacterial isolates were 4, 1 and 1 for *E. coli, Staphylococcus and Micrococcus* respectively. The result of this study also showed that *E. coli* was the most commonly isolated bacteria. The majorities of these isolates were resistant to the panel of antibiotic discs used in this study. The percentages of resistance were 100%, 83.33%, 100%, 83.33%, 100% and 100% for S, Te, C, T, P and SXT respectively. (Table.8).

Table.6: Results of biochemical tests used for identification of *Escherichia coli.*

Criteria	**Result**
Gram stain	-negative, small rod
MacConkey agar	Pink colonies
eosin-methylene blue agar	Colonies with green metallic sheen
citrate test	Negative
oxidase test	Negative
indole test	Positive
methyl red test	Positive
Voges-Proskauer test	Negative
catalase production	Positive
lactose fermentation	Positive
urea hydrolysis	Positive
nitrate Reduction	Positive
gelatin hydrolysis	Positive

Table.7: Shows the bacterial isolates from samples that collected from sheep and the resistances of bacteria to antibiotic discs.

Name of MO	**Isolated number**	SXT	P	T	C	Te	S
E.coli	25	20	24	23	21	24	20
Mannheimia haemolytica	6	6	6	6	6	6	6
Pasteurella Multocida	3	2	3	2	2	2	2
Klebsiell	2	2	2	2	2	2	2
Proteus	1	1	0	0	0	0	0
Salmonella	1	1	1	1	1	1	1
Total	38	32	36	34	32	35	31
Percentage		84.21%	94.7%	89.47%	84.21%	92.1%	81.57%

Table.8: Shows the bacterial isolates from samples that collected from sheep and the resistances of bacteria to antibiotic discs.

Name of MO	Isolated number	SXT	P	T	C	Te	S
E.coli	4	4	4	4	4	4	4
Staphylococcus	1	1	1	1	1	1	1
Micrococcus	1	1	0	1	0	1	1
Total	6	6	5	6	5	6	6
Percentages		100%	83.33	100%	83.33%	100%	100%

In samples collected from the cow, the number of bacterial isolates was 4, 1,1, 1 and 2 for *E.coli, Klebsiella, Pasteurella, Streptococcus* and *Proteus* respectively. The result of this study also shown that *E. coli* was the most commonly isolated bacteria. The majorities of these isolates were resistant to the panel of antibiotic discs used in this study. The percentages of resistance were 77.77%, 100%, 77.77%,100%, 100% and 77.77% for S, Te, C, T, P and SXT respectively (Table.9).

Table.9: Shows the bacterial isolates from samples that collected from sheep and the resistances of bacteria to antibiotic discs.

Name of MO	Isolated number	SXT	P	T	C	Te	S
E.coli	4	2	4	4	2	4	2
Klebsiella	1	1	1	1	1	1	1
Pasteurella	1	1	1	1	1	1	2
Streptococcus	1	1	1	1	1	1	2
Proteus	2	2	2	2	2	2	2
Total	9	7	9	9	7	9	7
Percentages		77.77%	100%	100%	77.77%	100%	77.77%

Only one E. coli isolate isolated from fecal samples of the cat with a different reaction to antibiotic discs panel, which was 11.52, R, 5.5, 12.1, 13.8 and 1 for S, Te, C, T, P, and SXT respectively (Figure.7).

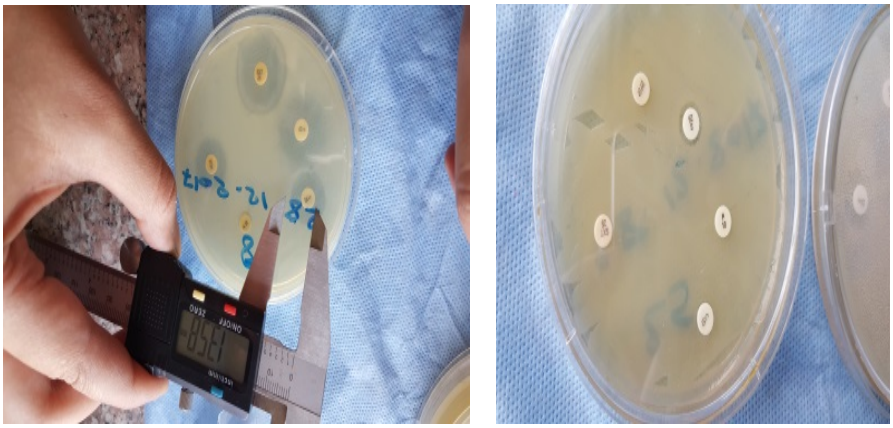

Figure.7: shows the antibiotic sensitivity test.

Tetracycline (tet) Resistance Genes

Ten *E. coli* isolates were resistance for tetracycline and oxytetracycline; these isolates were tested to identify the prevalence of tetracycline resistance genes tet (A). Nine out ten 9/10 (90 %) of *E. Coli* isolates were carried tetA gene (Figure.8). The tet (A) gene of strains was amplified by PCR with two sets of primers targeting the tetracycline efflux gene (tetA) (Figure. 8).

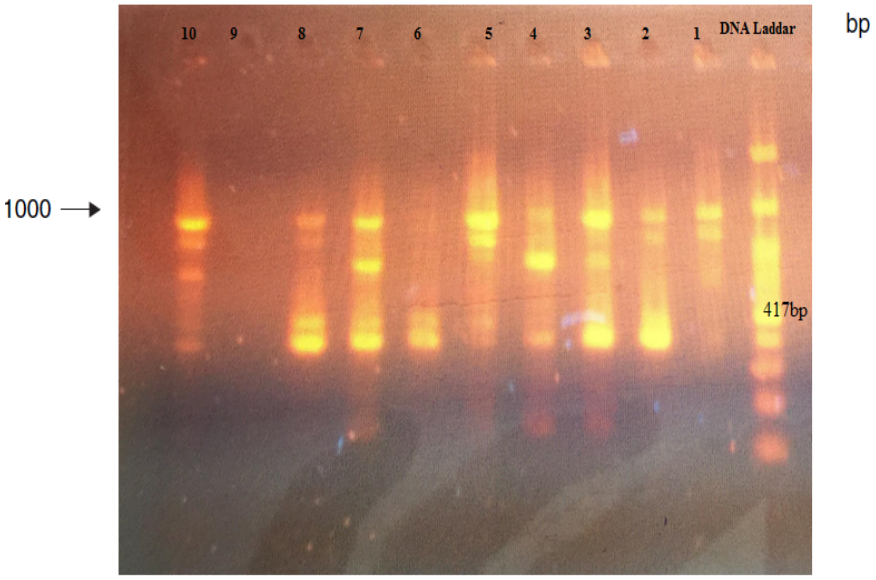

Figure.8: Strain genomic DNA profiles obtained with multiplex PCR. Shows the results for isolates of ten oxytetracycline/ tetracycline-resistant E. coli strains obtained from different animals. Multiple bands obtained from nine strain except isolates no. 9.

Discussion

The effective prevention, control, and prevention of dissemination of antibiotic resistance are depending on the basic understanding of the spreading, diversity of antibiotic-resistant bacteria and their resistance mechanisms. The mobile genetic elements such as plasmids, transposons or integrons (Davison 2002 & 1999; Rowe-Magnus *et al.,* 2002) are the place that carried the resistance determinants. The occurrence of horizontal gene transfer makes certain that antibiotic-resistant environmental strains deserve better investigations, especially in veterinary medicine, where the chance for humans to contact antibiotic-resistant bacterial contamination via consuming of animal product is high.

In this study, 53 samples including 9, 37, 6 and 1 were collected from cow, sheep, goat, and cat respectively. The total number of isolated bacteria were 53 isolates. The number of bacterial sheep isolates was 25, 6, 3, 1 and 1 for *E. coli, Mannheimia*

haemolytica, Pasteurella Multocida, Klebsiella, Proteus, and *Salmonella* respectively. Moreover, E. coli was the most commonly isolated bacteria. The majorities of these isolates were resistant to the panel of antibiotic discs and showed multip-bacterial resistance. The resistance of bacteria to tested antibiotic revealed high percentages 81.57%, 92.1%, 84.21%, 89.47%, 94.7%, 84.21% for S, Te, C, T, P and SXT respectively. Moreover, oxytetracycline and Tetracycline showed the highest percentages 92.1%, 94.7% respectively. These results are compatible with the results of the previous study that approved the presence of multiple-drug resistance from fecal samples of sheep (Lipsitch *et al.,* 2002).

The results of this study also approved the presence of resistant bacteria in goat. The number of bacterial isolates was 4, 1 and 1 for *E. coli, Staphylococcus*, and *Micrococcus* respectively. Meanwhile, this study also approved that *E. coli* was the most commonly isolated bacteria. Additionally, the majorities of these isolates were resistant to the panel of antibiotic discs used in this study. While the percentages of resistance bacteria reached to 100 % for some antibiotics. The percentages of resistance were 100%, 100%, 83.33%, 100%, 83.33% and 100% for S, Te, C, T,P and SXT respectively. These results are compatible with the previous study that reported the presence of multiple- resistance bacteria in the farm animals including goat (Chopra, Roberts, 2001). The samples and bacteria isolated from cow and goat also showed resistance to different types of antibiotics with variations in the percentages of resistance to each type of antibiotic. The number of bacterial isolated that investigated in cow was 4, 1,1, 1 and 2 for *E. coli, Klebsiella, Pasteurella, Streptococcus* and *Proteus* respectively. The result of this study also approved that E. coli was the most commonly isolated bacteria as the situation in sheep and goat. The majorities of these isolates were resistant to the panel of antibiotic discs used in this study. The percentages of resistance were 77.77%, 100%, 77.77%,100%,100% and 77.77% for S,Te, C, T, P and SXT respectively. Besides, this study showed that some isolates show 100% resistance to Te, C, T. This results are in agreement with previous studies that approved the isolation of antimicrobial resistant (AMR) bacteria from cow and its environments. This result is consistent with a previous study (Tamtam *et al.,* 2011).

Almost the massive number of *E. coli* isolated from animals were resistant to tetracycline and oxytetracycline because both are heavily used in the veterinary clinic for treatment, in addition to the unwittingly use. Moreover, the magnitude of resistance to an antibiotic is associated with the level of its use. The high antibiotic resistance rate of organisms isolated from animals is not a phenomenon unique to Iraqi animals, but it reported in previous reports. These found 100 % of farm E. coli strains were resistant to tetracycline and oxytetracycline. The present study findings are compatible with the increasing resistance of E. coli to antimicrobial agents in different countries worldwide (Rose & Pedersen, 2005). One report revealed multidrug resistance (MDR) in *E. coli* recovered from Irish cattle. *Daini and Adesemowo, (2008)* found the resistance *E. coli* clinical strains from Nigeria in 54 and 88% strains against gentamicin and tetracycline respectively, which is in agreement with the current finding. The high percentage of resistance to pefloxacin (88%) and amikacin (71%), which are rarely used in the farm animals, is raising a lot of questions as to why there is a high level of resistance to such antibiotics in natural non-clinical animals and how

the bacteria acquired resistance against the antibacterial. If these antibiotics are to be used, it is used only for treating bacterial infections not amenable to other commonly applied antibiotics such as enrofloxacin, ciprofloxacin, and gentamicin. MDR had been reported previously were in all isolates exhibited resistance to more than six antibiotics that did not differ from the findings of the present work.

In the present study, only one (10%) *E. coli* isolates did not show the presence of the tetA gene that agreed with the higher percentages of tetracycline- resistant isolates. It can be assumed that this tetA negative isolates reported in this study might be encoded by other genes such as tetB, tetC, and tetD or ribosomal protection encoded by tetM, tetO, tetQ and tetS genes than the gene monitored in this study. *Koo and Woo (2011*) have reported that 98.3% of meat-borne *E. coli* containing at least one of the tetA to tetD genes and was able to transfer tetracycline resistance to a tetracycline-susceptible recipient strain of *E. coli*. Interestingly, two isolates carried both tetA and tetB, but the only tetA was transferred to the recipient strain. It can be presumed that the tetA gene can be spread more easily in the environment than tetB. Antimicrobial resistance can spread to humans and animals via direct or indirect contact, consumed food/feed and through the environment. Therefore, it is essential to analyze the epidemiology and mechanisms of emergence and spread of antimicrobial resistance.

In conclusion, this study approved the presence of antimicrobial resistant (AMR) bacteria that isolated from different clinical cases refereed to Al-Muthanna veterinary hospital. Moreover, this study investigated the spread of resistant *E. coli* in Iraqi animals, with special emphasis on tetracycline and oxytetracycline resistant *E. coli*. This study also approved the presence of tetA resistance gene that found in 90% of the tested resistance *E. coli*. The Authors recommend doing another future study that includes a high number of bacterial isolates and determine other antimicrobial genes responsible for transferring the resistance between other kinds of bacteria. Besides, the authors recommend to use the antimicrobial wisely and prohibited providing this antibiotic without prescription.

References

Aarestrup, FM. (2004). Monitoring of antimicrobial resistance among food animals: principles and limitations. Journal of Veterinary Medicine B – Infectious Diseases and Veterinary Public Health 51:380–388.

Arzese AR, Tomasetig L and Botta GA. (2000). Detection of tetQ and ermF antibiotic resistance genes in Prevotella and Porphyromonas isolates from clinical specimens and resident microbiota of humans. J. Antimicrob. Chemother. 45(5): 577-582.

Blake DP, Humphry RW, Scott KP, Hillman K, Fenlon DR and Low JC. (2003). Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within commensal Escherichia coli populations. J. Appl. Microbiol. 94(6): 1087-1097.

Chansiripornchai N. (2009). Comparative efficacy of enrofloxacin and oxytetracycline by different administration methods in broilers after experimental

infection with avian pathogenic Escherichia coli. Thai Journal of Veterinary Medicine 39:231–236.

Carter GR. (1986). Essentials of veterinary bacteriology and mycology. 3rd edition. Philadelphia, Lea and Febiger.

Cizman M. (2003). The use and resistance to antibiotics in the community. International Journal of Antimicrobial Agents 21:297–307.

Chopra I. & Roberts M. (2001). Tetracycline Antibiotics: Mode of Action , Applications, Molecular Biology , and Epidemiology of Bacterial Resistance Tetracycline Antibiotics : Mode of Action , Applications , Molecular Biology , and Epidemiology of Bacterial Resistance. Microbiol. Mol. Biol. Rev. 65:232–260.

Clinical and Laboratory Standards Institute. (2009). *Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement M100-S19*, 2009 Wayne, PA Clinical and Laboratory Standards Institute.

Daini A O. and Adesemowo A. (2008). Antimicrobial Susceptibility Patterns and R-Plasmids of Clinical Strains of Escherichia Coli Australian Journal of Basic and Applied Sciences, 2(3): 397-400.

Davison J. (1999). Genetic exchange between bacteria in the environment. Plasmid 42: 73–91

Davison J. (2002). Genetic Tools for Pseudomonads, Rhizobia and other Gram-negative Bacteria: a Review. Biotechniques 32: 386–401

Ferens WA, Hovde CJ. (2011). Escherichia coli O157:H7: animal reservoir and sources of human infection. Foodborne Pathogens and Disease. 8:465–485.

Lipsitch M, Singer R S and Levin BR. (2002). Antibiotics in agriculture: When is it time to close the barn door? Proc. Natl. Acad. Sci. U. S. A. 99:5752-5754.

Koo H and Woo G. (2011). Distribution and transferability of tetracycline resistance determinants in Escherichia coli isolated from meat and meat products. Int. J Food Microbiol., 145(2-3): 407-413.

Mckellar QA, Sanchez Bruni SF, Jones DG. (2004). Pharmacokinetic/ pharmacodynamic relationships of antimicrobialdrugs used in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutics 27:503–514.

Mooljuntee S, Chansiripornchai P, Chansiripornchai N (2010). Prevalence of the cellular and molecular antimicrobial resistance against E. coli isolated from Thai broilers. Thai Journal of Veterinary Medicine. 40:311–315.

Li D, Liu B, Chen M, Guo D, Guo X, Liu F, Feng L, Wang L. (2010). A multiplex PCR method to detect 14 Escherichia coli serogroups associated with urinary tract infections. Journal of Microbiological Methods 82:71–77.

Riley LW, Remis RS, Helgerson SD, Mcgee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT, Blake PA, Cohen ML (1983). Hemorrhagic colitis associated with a rare Escherichia coli serotype. New England Journal of Medicine. 308:681–685.

Rose PE & Pedersen JA. (2005). Fate of oxytetracycline in streams receiving aquaculture discharges: model simulations. Environ. Toxicol. Chem. 24, 40–50.
Rowe-Magnus A. Dean, Guerout Anne-Marie, Mazel Didier (2002). Bacterial resistance evolution by recruitment of super-integron gene cassettes. Molecular microbiology. Volume 43;6:1657- 1669. https://doi.org/10.1046/j.1365-2958.2002.02861.x

Tamtam F, van Oort F, Le Bot B, Dinh T, Mompelat S, Chevreuil M, et al. (2011). Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term waste water irrigation. Sci. Total Environ. 409:540–547. 10.1016/j.scitotenv.2010.10.033