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Molecular Detection of Plasmid-Mediated Colistin-Resistance Genes in Multi-Drug Resistant *Escherichia coli* Isolated From Human and Poultry in Duhok City, Iraq

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THE plasmid-borne colistin resistance gene *mcr*-types were formerly recognized as plasmid-mediated colistin mechanism among clinical and animal samples *Escherichia coli* from China and thereafter reported worldwide. The study investigated for screening of plasmid-mediated colistin and carbapenems resistant genes in *Escherichia coli* isolates from human and poultry origin. After isolation and identification of isolates from clinical specimens and poultry cloacal swab, disc diffusion and broth microdilution method were determined antibiotic susceptibility patterns, phenotypic Extended-Spectrum Beta-lactamase enzymes (ESBL)-production test and phenotypic colistin resistance. Molecular detection by PCR was applied to targeted carbapenems-resistance genes (*KPC*-2*, OXA*-48 and *NDM*-1) and colistin-resistant genes (*mcr*-1, *mcr*-2 and *mcr*-3). The majority of isolates were higher resistance to commonly used antibiotic categories with Multi-Drug Resistance (63 %) and (93 %) of clinical and poultry *E coli* isolate, respectively. Using broth microdilution (16%) of isolates displayed phenotypic colistin resistance. Human isolates had higher *NDM*-1 gene (33.3%), while, higher *OXA*-48 gene (22.2%) was in poultry isolates, and no *KPC*-2 gene was identified. Only isolates from poultry contained the *mcr*-1 gene (27.7%), while *mcr*-2 gene was present only in human isolates (4.7%) and *mcr*-3 gene in both human (33.3%) and poultry (22.2%) isolates. Negative *mcr*-1, 2, and 3 isolates expressed high rates of ESBL-production. The coexistence of *(mcr*-3 + *NDM*-1) was frequent, including 10 isolates, and one isolate carried (*mcr*-2+*mcr*-3+*NDM*-1) combination from urine samples. Data of colistin resistance among clinical and poultry isolates in this region is scarce. Identification of colistin and carbapenems- resistance genes via plasmids among those isolates menace and approaches pan-resistance and failure treatment.

Keywords: *Escherichia coli,* Colistin, Carbapenems, Poultry, PCR.

Introduction

Among the serious infections frequently brought by *Enterobacteriaceae,* specifically *Escherichia coli* is a real public health concerns [1]. Therefore, antibiotic resistance in these microorganisms has significant clinical and socioeconomic effects [2]. Carbapenems were once regarded as the most effective antibiotics for treating severe infections caused on by *Enterobacteriaceae* that are multidrug resistant. Although polymyxins are already available antibiotics with relatively high nephrotoxicity, they have gained appeal as a last resort for the treatment of clinical carbapenem-resistant *Enterobacteriaceae* (CRE) infections as the number of (CRE) strains has increased [3]. The administration of polymyxins, an antibacterial drug mostly abandoned by the middle of the 1970s due to toxicity, has increased as carbapenem-resistant gram-negative bacteria have become more prevalent worldwide [4]. Colistin is

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currently used to treat infections in humans and animals, which has resulted in the discovery of an increasing number of colistin and drug resistance mechanisms [5]. This resumption is related to the rise of organisms that are resistant to polymyxin [6].

The mobile colistin resistance-1 (*mcr*-1) gene, a plasmid-mediated mechanism of colistin resistance, was discovered in *Enterobacteriaceae* recovered from food, animals, and human specimens in China in November 2015, and it has since been reported everywhere [7]. An unusual variation of the *mcr* gene is *mcr*-2. The *mcr*-1 and *mcr*-2 genes are found on conjugative plasmids, which could facilitate the spread to other strains and bacterial species and cause the therapeutic regimen to fail [8]. In accordance with findings on the mcr-1 and mcr-2 genes, *Escherichia coli* resistance has increased [9]. Numerous investigations have documented the presence of variations such as *mcr*-3, *mcr*-4, and *mcr*-5 in isolates of *Salmonella* and *E. coli* [10]. The transmission of colistin resistance between animals and human via the *mcr*-1 gene carries potential health risks. Additionally, *Klebsiella pneumonia* in China has been found to harbor the mcr-7.1 colistin resistance gene [11]. Colistin resistance caused by the *mcr* genes should be of great concern to doctors as it is one of the few available therapeutic choices against CRE [12]. Recently, the emergence of the plasmid-mediated carbapenems and colistin-resistance gene represents a great concern to global public health. The spreading of *mcr*–types in to CRE means there will be little and/or no antibiotic available for the infections caused by such strains [13] However, the majority of *mcr*-genes-related studies focus on monitoring colistin-resistant bacteria, and it is still unclear how prevalence of the *mcr* genes is found in E. coli isolates in this setting. Therefore, we undertook this experiment in order to determine the prevalence of the carbapenem-resistant genes (*KPC*-2, *OXA*-48 and *NDM*-1) and colistin-resistant genes (*mcr*-1, *mcr*-2 and *mcr*-3) among *Escherichia coli* strains from human clinical specimens and healthy poultry origin in Duhok City, Iraq.

Material and Methods

Bacterial Strains

This cross-sectional investigation enrolled a collection of 150 *E. coli* isolates including110 human clinical isolate and 40 poultry origin isolates that gathered from December 2020 to the end of April 2021. Human clinical isolate comprised of (urine=85), (wound swabs =12, (blood cultures =2), (sputum =2), (vaginal swab =7) and (semen fluid samples =2). Those clinical isolates were obtained from three governorate hospitals and one central health lab in Duhok, Iraq, the patients were either admitted as in-patients or visited clinic departments (out-patient). Poultry cloacal swabs (n=40) were collected from chickens in the Duhok abattoir in Duhok city, Iraq, served as the veterinary samples (poultry origin). Regarding clinical samples, every participant gave informed written consent prior to the study starting, and the confidentiality of the data collected was guaranteed. The study's methodology was approved by the ethics committees of Duhok University and Duhok General Health Directorate, per protocol ET-P 8/11/2020.

Sample processing and identification

With using a sterile loop, all samples (human and poultry) were streaked on Blood agar, MacConkey agar, and chrome agar medium (Oxoid UK). The samples were then incubated at 37 ºC for 24-48 hours. Isolates were only included after a pure culture with > 10^5 CFU/ml was obtained from urine samples that were cultured using a standard sterile calibrated loop. Regarding blood culture, adult patients' 5 mL of blood were drawn into sterile syringes, suspended in 45 mL of brain heart infusion broth (Oxoid, UK), and then incubated at 37 ºC for 5-7 days[14]. All strains were first identified by traditional biochemical tests (Gram staining, oxidase, IMVIC, and Triple Sugar Iron Agar) then subjected to the VITEK-2 Compact system. Further accomplish of specie-level confirmation identification was achieved through *uid*A gene amplification using the primers listed in Table (1) [14].

Antibiotics-susceptibility patterns

The agar disk diffusion technique was used to test the bacteria's susceptibility to a panel of 16 antibiotics from various classes, including the including Ampicillin(Am), Amoxillin+clavulanic acid (AMC), Piperacillin-tazobactam (TZP), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefepime(FEB), Cefoxitin(CFM), Cefotaxime (CTX), Meropenem(MRP), Imipenem(IMI), Gentamicin(CN), Tetracyclin (TE),Trimethoprim/ Sulphamethoxazole (SXT), Chloramphenicol(C), Ciprofloxacin(CIP) and Colistin(CS) were tested using the agar disk diffusion method [15, 16]. Using

sterile swabs, pure cultures of known bacteria were suspended and then dispersed on Muller Hinton agar (Oxoid, UK) media using a 0.85 % saline to 0.5 McFarland turbidity criteria. Instructions for interpreting the findings were provided by the Clinical and Laboratory Standards Institute CLSI 2017. On a qualitative level, the majority of the data was categorized as resistant, intermediate, or susceptible. To determine the colistin's Minimum Inhibitory Concentration (MICs), reference broth microdilution was used. Briefly, the isolated pure bacterial cultures were inoculated into a series of Mueller-Hinton broths containing 2-fold dilutions of colistin antibiotic in 0.01% acetic acid and 0.4% BSA (bovine serum albumin). The turbidity standard that was applied was 0.5 McFarland. The lowest antibiotic concentration that, after being incubated at 37 ºC for 18–24 h, completely inhibited bacterial growth was known as the MIC. According to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints (http://www.eucast.org/clinical_breakpoints/), isolates with (MIC) 2 µg/ml were considered to be colistin-resistant**.** Isolates were also subjected to polymerase chain reaction (PCR) using published primers [22] to ascertain whether particular strains contained the (*mcr*-types) genes. Isolates that have been found to be resistant to three or more antimicrobials from different classes are known as multi-drug resistant (MDR) isolates [14].

Phenotypic detection of Extended-Spectrum β-lactamase (ESBL) production

All isolates underwent a double disc synergy test (DDST) with four antibiotics: amoxicillin/clavulanic acid (AMC, 20/10 g), cefotaxime (30 g), ceftazidime (30 g), and aztreonam (ATM, 30 g) to determine whether they produced (ESBLs) as described by Rhouma et al. [17].

Preparation of DNA templates for PCR testing

The selection of 60 *E. coli* isolates (42 from humans and 18 from poultry) was made in consideration of the variety of sample sources, ESBL production, carbapenems and colistin susceptibility patterns. Subsequently were further subjected to PCR assay for screening of plasmid mediated carbapenems and colistin- resistance genes. For genomic extraction, heat shock method was used and lysate crudes were used as templates for PCR assays as described by Yang et al. [18] as follows: the bacterial cells were lysed by boiling at 100 ºC for 20 min (in a water bath) using five pure, fresh colonies suspended in 200 µL of dry weight. The other cellular components were separated by centrifuging them at 9000 rpm for 10 minutes after they had been placed in ice for 40 minutes. The DNA template was then created using the supernatant. All the 60 isolates were checked for genus specific gene and for the carbapenems resistance genes (*KPC*-2*, OXA*-48 and *NDM*-1*)* and colistin resistant genes (*mcr*-1*, mcr*-2 and *mcr*-3) using the corresponding primers given in Table (1). PCR was performed in 30 µL reaction volumes containing 3 µl of 10 × buffer [100 mM Tris-HCl (*p*H 9), 1.5 mM $MgCl_2$, 50 mM KCl and 1% gelatin, 100 µM of four deoxyribonucleotide triphosphates each (dATP, dGTP, dCTP and dTTP), 10 pmol of each forward and reverse primers and 1.0 U of *Taq* DNA polymerase with 2 µL of template DNA. All the isolates were tested for the presence of PMQR genes using primers listed in (Table 1). Gel staining, DNA safe staining, and electrophoresis on a 1 % agarose gel were all performed on 10-ml aliquots of the PCR product. The amplicon sizes on the gel electrophoresis were determined by comparing them to a 100 (bp) DNA marker. Amplified fragments of DNA with a particular molecular weight have been The selection of 60 *E. coli* isolates (42 from humans and 18 from poultry) was made in consideration of the variety of sample sources, ESBL production, carbapenems and colistin susceptibility patterns. Subsequently were further subjected to PCR assay for screening of plasmid mediated carbapenems and colistin- resistance genes. For genomic extraction, heat shock method was used and lysate crudes were used as templates for PCR assays as described by Yang et al. [18] as follows: the bacterial cells were lysed by boiling at 100 ºC for 20 min (in a water bath) using five pure, fresh colonies suspended in 200 µL of dry weight. [19].

Statistical analysis

The study sample's data analysis was described using means, standard deviations, frequencies and percentages. To analyze the data, SPSS v23 (SPSSInc, Chicago, IL, USA) was utilized [24].

Results

Clinical samples from patients of all ages, including those with UTIs (n = 85), wound infections

(12), blood samples (2), sputum (2), vaginal swabs (7), and semen fluid samples (n = 2), yielded 110 *E. coli* isolates. From another hand, 40 poultry *E. coli* isolates were recovered from cloacal swabs of healthy chickens. All of those isolates that confirmed by phenotypic tests were confirmed as *Escherichia coli* species by PCR-based genotypic test for amplification of *uid*A gene**.**

Antibiogram analysis

All human and poultry strains generally exhibited a significant resistance to penicillins, cephalosporins, aminoglycosides, and flouroquinolon es, with resistance to ampicillins (86 - 97 %), ceftriaxone (74 - 47 %), tetracycline (72 - 85 %), and ciprofloxacin (48 - 97 %) respectively. The lowest resistance proportions were found for imipenem and meropenem (4 - 2 %) respectively, indicating that carbapenems were the most effective antibiotic (Table 2). According to our findings, 69 (63 %) and 37 (93 %) of the *Escherichia coli* isolates from clinical and poultry samples, were MDR, respectively. At this point, of the 140 human and poultry isolates, 24(16%) isolate had phenotypic colistin resistance accounted (17-12%), respectively.The minimum inhibitory concentration (MIC) for these colistin-resistant isolates using broth microdilution was ≥2-64 µg/ml, which corresponds to EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoints (http://www.eucast.org/clinical_breakpoints/) (Table 3).

Molecular detection of carbapenems and colistin-resistant genes

Results of plasmid-mediated carbapenems resistance genes (PMCA) identified by PCR using (*KPC*-2, *OXA*-48 and *NDM*-1) primers revealed that human isolates had more *NDM*-1 gene (33.3%), while poultry isolates harbored more *OXA*-48gene (22.2%). None of the investigated isolates were found to carry *KPC*-2 gene. Among colistin-resistance gene (PMCO); only poultry isolates carried the *mcr-1* gene (27.7%), while only human isolates (4.7%) carried the *mcr*-2 gene. Clinical isolates had higher percentages of the *mcr-3* gene (33.3%) than poultry isolates (22.2%). (Table 4). Negative-*mcr*-1, 2, and 3 isolates showed remarkable ESBL-production; however, only positive-*mcr*-3 human clinical isolates (21.7%) were have been found ESBL producers (Table 5). Patterns of co-existence carbapenems and colistin-resistance genes found in 13 isolates and combination of *(mcr*-3 + *NDM*-1) was more frequent included 10 isolates (76.9%) in human and poultry sources. Additionally, triple gene combination (*mcr*-2+*mcr*-3+*NDM*-1) was occurred one time belong urine isolates (Table 6).

Discussion

Detection of *Escherichia coli* antimicrobial resistance patterns from various origins are crucial for understanding epidemiological data, allowing an alternative treatment options and optimizing excellent infection control methods[25]. The percentage of human and poultry isolates in this study that had MDR was (63% – 93%) respectively. Subsequently, according to the study's findings, ampicillin, amoxicillin-clavulanic acid, ceftazidime, trimethoprim-sulfamethoxazole, cefioxitine, ceftriaxone, cefepime, and cefotaxime were most ineffective. Our data is in accordance with other studies such as [26, 27]. High MDR rates in poultry strains were supported by other studies mentioned poultry *Escherichia coli* isolates had higher levels of antibiotic resistance than beef isolates [28, 29]. Frequent use of those antibiotics as empirical therapy in domestic/companion animals and agricultural fields as well as production of ESBLs enzymes and the efflux pump mechanism might contribute to the resistance[30, 31]. Ciprofloxacin showed good *in vitro* activities and resistance was relatively moderate (48 %) in human isolates but extreme high (97%) in poultry isolates in our results. Overuse of quinolones in veterinary medicine as a growth promotor and bacterial biofilm during UTI treatment suggested reasons of resistance[32, 33]. Our data is in agreement with other investigations[34, 35] Carbapenems such as imipenem and meropenem were efficient drugs as resistance rates in humans and poultry were low (4-2%), respectively in this study. These antibiotics are may be appropriate options and successful in treating *Escherichia coli* strains once, but due to self-medication and excessive usage, both of which are quite common in this region, resistance is growing. This finding is in line with previous report [34], while dissimilar to

Majewski et al. [36] Our patients have not had history of colistin therapy but it is currently prescribed by veterinaries. Overall, 24(16%) isolate had phenotypic colistin resistance of them 19 (17%) clinical sitting isolate from outpatients. This worrisome finding essentially is high when compared to Taiwan, 3 out of 420 isolate were colistin-resistant[37]. and in Italy, 18 out of 3,902 (0.5%) isolates expressed colistin-resistance[38], when considering only outpatients taking into account enforcing the major high risk sources outside the health-care setting such contact with animals and environments. These sources may have transferable resistance determinant factors, as we found in current study that (12%) of healthy poultry isolates exhibited phenotypic colistin resistance. So large-scale study is further need to assess a multi-variegate source of infections. The first chines description study of *mcr*-1 from animals and human confirmed that the levels of maximum inhibitory concentrations of polymyxin conferred by *mcr*-1 were not very high (4–8 mg/L), but presence of *mcr*-1 provided adequate protection from colistin [39]. Since then *mcr*-1 has attracted global attention to identifying it in isolates from humans, animals and the environment in an increasing number[40]. Fortunately, none of the human isolates examined in our study showed positive for *mcr*-1, which is consistent with very low background carriage of *mcr*-1 in humans as previously reported [41-44] Dissimilarly, (5.3%) of the colistin resistant clinical *Escherichia coli* strains from Arabian Peninsula carried the *mcr*-1 gene[45, 46]. In contrast, *mcr*-1 was detected in two clinical carbapenem-resistant *Escherichia coli* strains recovered from urine cultures from the United States acquired in April 2016[47] and May 2015[48], respectively In Italy, the *mcr-1* gene was detected in 10/18 isolates from outpatients[49]. In the current investigation, *mcr*-1 was solely found in (25%) of the chicken isolates. Two studies in China, first found (52.5%) of clinical *Escherichia coli* isolates were harbored *mcr*-1[50]. Second study observed 166/804 (21%) and 16/1322 (1%) of *mcr*-1 carriage *Escherichia coli* isolates from animals samples and inpatients with infections, respectively[51]. Beforehand, a retrospective study of 1611 chicken farms-*E.coli* isolates revealed that the earliest isolate was possessed *mcr*-1 due to China's policy of giving colistin to livestock in the 1980s[52]. The finding of *mcr*-1 in poultry isolates but not in clinical isolates in our investigation was anticipated, since colistin has been and is currently routinely used in veterinary medicine, putting chicken retails and food animals at high risk. Despite, low sampling size of our study making it likely that it is not representative but demonstrates that *mcr*-1-mediated colistin resistance started in animals and then will expand to human[53].

Here, we further examined a colistin-resistant *E. coli* isolates for carrying both *mcr*-2 and *mcr*-3 gene and discovered that exclusively clinical isolates (4.7%) harbored *mcr*-2 gene as well as higher percentages of *mcr*-3 gene (33.3%) compared to poultry isolates that had (22.2%) of *mcr*-3 gene. Our results are in parallel with *mcr*-1 while being inverse with *mcr*-2 regarding assumption concept, which is in accordance with the fact that *mcr*-1 and *mcr*-2 are dominant in animals and were initially discovered in animals that ingest the most colistin. [54]**.** Moreover, our findings suggest that *mcr*-3 is a mechanism of underline plasmid mediated-colistin resistance in majority of clinical isolates of these results. Further research is required to determine the function of the novel *mcr*-3 genes in clinical *Escherichia coli* isolates from human infections. Imported food, environments, mass gatherings like annual Hajj[54] and travelers highlight the potential for *mcr*-3 to continue spreading. Additionally, occupations of our patients notably farmers are strongly associated with spread of all types of *mcr*-genes, albeit some farmers use colistin to improve the quality of food animals[55]. Other study indicated that the *mcr*-3 gene was already present in at least three *Enterobacteriaceae* species (*Escherichia coli, K. pneumonia* and *Salmonella enterica*) in both agricultural and clinical settings in Southeast Asia and North America[56]. Disagreement, in Belgium, *mcr*-2 (21%) was prevalent among bovine porcine colistin-resistant Escherichia *coli* [56] . Further research is required to determine the function of the *mcr*-3- genes in clinical E coli isolates from human infections[57].

When it comes to the extended-spectrum beta-lactamases (ESBLs) phenotypic production and *mcr* genes among 42 positive and negative-*mcr*-1, 2 and 3 clinical isolates in current study, intriguingly, the majority of the negative *mcr*-isolates were ESBL producers, whereas only (21.7%) of the positive *mcr*-

3 isolates were. Meanwhile, co-production of ESBLs by *Enterobacteriaceae* that harbor *mcr*-1 has now been confirmed worldwide [52, 58]. One study highly emphasized on travelling abroad in regard to colonization with *mcr*-1 and ESBL-enzyme producing *Escherichia coli*[40]. This suggests that commonly utilized beta-lactam antibiotics may also co-select for such a type of plasmid.[55] In this study, human isolates had more *NDM*-1 gene (33.3%), while poultry isolates harbored more *OXA*-48 gene (22.2%). Among co-existence of carbapenem resistance and *mcr*- resistance genes simultaneously in our data, 13 isolates showed co-existence, (*mcr*-3 + *NDM*-1) was predominant included 10 isolates (76.9%) of human and poultry sources. Interestingly, the three genes (*mcr*-2+ *mcr*-3+ and *NDM*-1) combination occurred once in isolates of urine. This finding in this region is of grave concern approaches a pan-drug-resistant phenotype; rendering treatment of infection cases is ineffective. Thereby, co-localization stresses inquiries about antibiotic stewardship across the One Health platform not only for antibiotics of last resort like colistin but also to commonly used antibiotics. There has been a numerous case worldwide of *mcr*-types co-localizing with carbapenemases genes. In current study, no *mcr-1* and *NDM*-1 was recorded in clinical sitting, while it's reported in clinical *E coli* isolates from the Arabian Peninsula and one isolate carried the bla*NDM*-1 carbapenemase gene [56]. In Venezuela by Delgado et al., 2016[56] found *mcr*-1 gene in 2/93 *E.coli* isolates from swine and human samples and one of the isolates bored the *NDM*-1. A comparable observation was obtained from a patient with a urinary tract infection in the USA [57]. In our study, we did not find association of *mcr* and *blaKPC*-2 co-existence, while other studies found this association [52, 58]. In this regard**,** we detected (*mcr*-1 and *OXA*-48) co-existence solely among poultry isolates; in contrast a Canadian study found it in gastrostomy tube site and rectum of a Canadian patient had lived in Egypt for 5 years[12].

Conclusion

All human and poultry strains generally exhibited a significant resistance to numerous classes of commonly used antibiotics except carbapenems were the most effective antibiotic. With great concern more MDR were among poultry isolates. Regardless of phenotypic carbapenems efficacy, but carbapenems-resistance genes are circulating among our strains. Prevalence data on colistin resistance are overall scarce in Duhok city, in particular, data regarding the plasmid-mediated colistin-resistance among poultry and clinical isolates of *E. coli.* Reporting the emergence of (*mcr*-1, *mcr*-2 and *mcr*-3) is of concern because this drug is one of the last effective drugs for the treatment of multidrug-resistant Gram-negative infections. Fortunately, *mcr*-1 was not detected in clinical sitting isolates; however, *mcr*-3 was exclusive determined gene and suggested a key of colistin resistance. Presences of co-existence carbapenems-resistance and colistin-resistance gene are of grave concern and approaches for pan-drug resistance to be expected circulating and spreading in this sitting. Reduce indiscriminate prescribing of frequently used antibiotics that could lead to generation of a selective pressure and emergence of colistin-resistant genes if they are co-existed. Therefore, it's crucial to prevent the transmission of *mcr*-genes, especially among strains that are already resistant to carbapenems.

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Conflict of Interest

According to the authors, there is no conflict of interest.

Ethical Approval

The ethics committees of Duhok University, Duhok General Health Directorate, and College of Medicine have all given their approval to the study protocol, which has the protocol identification number ET-P 8/11/2020.

TABLE 1. Primers used for detection of carbapenem-resistance genes and colistin-resistance genes

Primers	Sequence 5' → 3'	Amplicon size (bp)	Annealing Temp (℃)	Reference
***uid*A**	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTACAGTCTTGCG	147	58	[20]
	Carbapenem-resistance genes			
***KPC*-2**	F: TTGCCGGTCGTGTTTCCCTTTAGC R: GGCCGCCGTGCAATACAGTGATA	282	64	[21]
***OXA*-48**	F: CGCCCGCGTCGACGTTCAAGAT R: TCGGCCAGCAGCGGATAGGACAC	484	63	[21]
***NDM*-1**	F: CTTCCAACGGTTTGATCGTC R: TTGGCATAAGTCGCAATCC	208	56	[22]
	Plasmid mediated Colistin-resistance genes			
***mcr*-1**	F: 5′-ATGCCAGTTTCTTTCGCGTG-3′ R :5′-GGCAAATTGCGCTTTTGGC-3′	502		
***mcr*-2**	F 5′-GATGGCGGTCTATCCTGTAT-3 R 5 AAGGCTGACACCCCATGTCAT-3′	379	59	[23]
***mcr*-3**	F 5′-ACCAGTAAATCTGGTGGCGT-3 R 5′-AGGACAACCTCGTCATAGCA-3′	296		

TABLE 2. Antibiotics susceptibility patterns of clinical and poultry *Escherichia coli* isolates

Antibiotics	**Human No. (%) (110 isolate)**			**Poultry No. (%) (40 isolate)**		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Ampicillin (Am)	95 (86)	0 (0.0)	15 (13)	39 (97)	1 (2)	0 (0)
Amoxillin+clavulanic acid (AMC)	61 (55)	16 (14)	33 (300)	26 (65)	13 (32)	1 (2)
Piperacillin-tazobactam (TZP)	25 (23)	10 (9)	75 (68)	2 (5)	0 (0)	38 (95)
Ceftriaxone (CRO)	82 (74)	0 (0.0)	28 (25)	19 (47)	1 (2)	20 (50)
Ceftazidime (CAZ)	84 (76)	0 (0.0)	26 (24)	12 (30)	2 (5)	26 (65)
Cefepime (FEB)	83 (75)	0 (0.0)	27 (25)	11 (27)	4 (10)	25 (62)
Cefoxitin (CFM)	53 (48)	5 (4)	52 (47)	6 (15)	5 (12)	29 (72)
Cefotaxime (CTX)	73 (66)	1 (0.9)	36 (33)	10 (25)	3 (7)	27 (67)
Meropenem(MRP)	4 (3)	0 (0.0)	106 (96)	1 (2)	0 (0)	39 (97)
Imipenem (IMI)	5 (4)	1 (0.9)	104 (94)	1 (2)	0 (0)	39 (97)
Gentamicin (CN)	37 (33)	0 (0.0)	73 (66)	22 (55)	0 (0)	18 (45)
Tetracyclin (TE)	79 (72)	1 (0.9)	30 (27)	34 (85)	1 (2)	5 (12)
Trimethoprim/ Sulphamethoxazole (SXT)	59 (54)	1 (0.9)	50 (45)	25 (62)	0 (0)	15 (37)
Chloramphenicol(C)	33 (30)	2 (2)	75 (68)	30 (75)	0 (0)	10 (25)
Ciprofloxacin (CIP)	53 (48)	2 (2)	55 (50	39 (97)	0 (0)	1 (2)
Colistin (CS)	19 (17)	7 (6)	84 (76)	5 (12)	7 (17)	28 (70)

TABLE 3. Patterns of colistin-susceptibility in clinical and poultry *Escherichia coli* isolates

Colistin	Breakpoint (µg/ml)	Resistant	Susceptible	Clinical Isolates (110)	Poultry Isolates (40)	MIC (µg/ml)
	Susceptible < 2	0	126 (84%)	91 (83%)	35 (88%)	1-64
	Resistant ≥ 2	24 (16%)	0	19 (17%)	5 (12%)	

TABLE 4. Distribution of plasmid mediated carbapenems and colistin-resistant genes of clinical and poultry *Escherichia coli* isolates

Genes	No (%)				p-value
	Human isolates		Poultry isolates		
	Negative	Positive	Negative	Positive	
KPC-2	42 (100)	0 (0.00)	18 (100)	0 (0.0)	0.08
OXA-48	39 (92.8)	3 (7.1)	14 (77.7)	4 (22.2)	0.0954
NDM-1	28 (66.6)	14 (33.3)	16 (88.8)	2 (11.1)	0.0744
mcr-1	42 (100)	0 (0.0)	13 (72.2)	5 (27.7)	0.0003
mcr-2	40 (95.2)	2 (4.7)	18 (100)	0 (0.0)	0.3463
mcr-3	28 (66.6)	14 (33.3)	14 (77.7)	4 (22.2)	0.3894

Pearson chi-squared tests were performed for statistical analyses.

TABLE 5. Association of Co-ESBL production with colistin genes

Genes	ESBL-production		p-value (two-sided)
	Negative (n=19)	Positive (n=23)	
mcr-1			
Negative isolates	19 (100)	23 (100)	NA
Positive isolates	0 (0.0)	0 (0.0)	
mcr-2			
Negative isolates	17 (89.4)	23 (100)	0.1986
Positive isolates	2 (10.5)	0 (0.0)	
mcr-3			
Negative isolates	10 (52.6)	18 (78.2)	0.1067
Positive isolates	9 (47.3)	5 (21.7)	

Pearson chi-squared tests were performed for statistical analyses.

TABLE 6. Co-existence patterns of carbapenems and colistin resistance genes

Genes patterns	No. (%)	Source
mcr-1+*OXA*-48	1 (7.7)	Poultry
mcr-3+*OXA*-48	1 (7.7)	Human
mcr-3 +*NDM*-1	10 (76.9)	Both sources
mcr-2+*mcr*-3+*NDM*-1	1 (7.7)	Human
Total	13 (100)	

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الكشف الجزيئي عن الجينات المقاومة للكوليستين بوساطة البلازميد في جرثومه الإشريشية القولونية *Escherichia coli* المقاومة للأدوية المتعددة والمعزولة من الإنسان والدواجن في مدينة دهوك ، العراق

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تم التعرف سابقًا على أنواع *mcr* الجينية المقاومة للكوليستين المنقولة بالبلازميد في العينات الحيوانية والسريرية من البكتريا المعوية فى الصين وتم الإبلاغ عنها بعد ذلك في جميع أنحاء العالم. الهدف هنا هو فحص عزلات الإشريشية القولونية السريرية والدواجن للكشف الجزيئي للجينات المقاومه للكاربابينيمات والكولستين والتي تتوسط البلازميد. وعليه تم تحديد أنماط الحساسية للمضادات الحيوية ، واختبار إنتاج انزيم ESBL بالنمط الظاهري ومقاومة الكوليستين المظهرية لعزلات *Escherichia coli* من العينات السريرية البشرية والدواجن السليمة في مدينة دهوك ، العراق بواسطة طريقة نشر القرص وطريقة التخفيف الدقيق للمرق broth microdilution. تم تطبيق الكشف الجزيئي بواسطة PCR على جينات مقاومه لمضاد الحيوى كاربابينيم و المستهدفة منها الجين (*KPC*-2 و *OXA*-48 و *NDM*-1) والجينات المقاومة للكوليستين منها الجين (*mcr*-1 و *mcr*-2 و *mcr*-3). كانت غالبية العزلات ذات مقاومة أعلى لفئات المضادات الحيوية شائعة الاستخدام وذات المقاومة للأدوية المتعددة (63٪) و (93٪) من العزلات البشريه وعزلات الدواجن ، على التوالي. أظهرت استخدام مرق دقيق (16٪) من العزلات مقاومة كوليستين المظهرية. كانت العزلات البشرية تحتوي على جين *NDM*-1 أعلى (33.3٪) ، بينما كان أعلى جين 22.2) *OXA*-48٪ في عزلات الدواجن ، ولم يتم تحديد جين *KPC*-2نهائيا ضمن العزلات. تم العثور على جين 27.7) *MCR*-1٪ حصريًا في عزلات الدواجن ، بينما وجد جين *mcr*-2 فقط في العزلات البشرية (4.7٪) وجين *mcr*-3 في العزلات البشرية (33.3٪) والدواجن (22.2٪). أظهرت العزلات السالبه ل *mcr*-1 و 2 و 3 معدلات عالية من إنتاج انزيم ESBL . كان تعايش (*NDM*-1 + *mcr*-3) متكررًا ، متضمنًا 10 عزلات ، وعزلة واحدة محمولة (*mcr*-2 + *NDM*-1 + *mcr*-3) من عينات البول.المعطيات المقاومة الكوليستين بين العزلات السريرية والدواجن في هذه المنطقة شحيحة. الكشف عن جينات مقاومة الكاربابينيم والكوليستين التي تتوسط البلازميد بين تلك العزلات خطرة وتقترب من المقاومة الشاملة و فشل العلاج.

الكلمات المفتاحيه: بكتريا الاشريشيه القولونيه ، كوليستين ، كاربابينيم ، تفاعل سلسلة المتضاعف.