Palestinian Medical and Pharmaceutical Journal (PMPJ). 2024; 9(2): 157-170

Colistin and Antimicrobial Resistance among Gram-Negative Bacteria Isolated from Poultry in West Bank, Palestine

Mohammad Qadi[1,]; Rasha Khayyat[1]; Safaa Alhato[2]; Abdelraouf Elmanama[2]; Ashraf Zayed[1]; Muna M. Abbas[1,3]; Maysa Daqqa[1]; Faizeh Hussein[1] & Ahmed Mousa[1]*

[1]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine. [2]Department of Medical Laboratory Sciences, Faculty of Health Sciences Islamic University of Gaza, Gaza Strip, Palestine. [3]Department of Allied Medical Sciences, Zarqa University College, Al-Balqa Applied University, Zarqa, Jordan.

*Corresponding author: m.qadi@najah.edu

Received: (29/4/2023), Accepted: (13/8/2023), Published: (1/6/2024)

ABSTRACT

Background: The escalating incidence of antimicrobial resistance in Gram-negative bacteria has elicited worldwide apprehension owing to its profound ramifications, such as the emergence of infections that cannot be effectively treated and may result in mortality. The poultry industry is of significant concern due to its substantial utilization of antimicrobials, including those regarded as last-resort therapies for complex multi-drug resistant Gram-negative infections. **Methods:** A comprehensive collection of ninety cloacal swabs was undertaken from a diverse range of thirty poultry farms located within the Nablus governorate during the period spanning from March to June in the year 2019. The swabs, specifically collected from broilers, were cultured on appropriate culture media to isolate Gram-negative bacteria under optimal conditions. The separated bacteria were subjected to various microbiological techniques, such as Gramme staining, oxidase testing, and API20E, to aid in their identification. The susceptibility of the isolates for 16 antibiotics was evaluated using the disc diffusion method, while colistin was assessed using the micro-broth dilution method. Additionally, molecular characterization was conducted using polymerase chain reaction (PCR) to identify the existence of *MCR* genes. **Result:** A total of 244 isolates of Gram-negative bacteria were collected and subjected to characterization, identification, and categorization into two groups: the Enterobacteriaceae group consisting of 170 isolates (69.7%) and the remaining 74 isolates (30.3%) categorized as others. The initial cohort consisted of 103 Escherichia coli (42.2%), 24 Proteus mirabilis (9.8%), and 19 Salmonella spp. (7.8%). The second group consisted of 62 Serratia spp. (25.4%), 1 Aeromonas hydrophila (0.4%), and 11 other unidentified species (4.51%). The study observed varying levels of antibiotic resistance among different antibiotics. The highest resistance rates were found for trimethoprim (100%), tetracycline (94.3%), trimethoprim-Sulfamethoxazole (90.6%), ciprofloxacin (86.1%), chloramphenicol (80.7%), ampicillin (75.4%), gentamicin (50.4%), fosfomycin (17.2%), cefuroxime (10.2%), cefotaxime (10.2%), ceftriaxone (7%), ceftazidime (2%), amikacin (1.2%), and meropenem (0.4%). Furthermore, the percentages of multidrug-resistant (MDR), extensively drug-resistant (XDR), and Extended-spectrum beta-lactamase (ESBL) isolates were 83.8%, 4.8%, and 18.6%, respectively, with corresponding proportions of 140/167, 8/167, and 31/167. Notably, a significant proportion of the total isolated bacteria, specifically 65.4%, exhibited resistance to colistin as determined through the micro-broth dilution method. In our study, 167 Enterobacteriaceae isolates were examined, of which 111 (66.5%) were resistant to colistin. Among these colistin-resistant isolates, 35 (31.5%) were found to possess variants of the *MCR* gene. The gene variant *MCR*-7 was the most prevalent among the isolates, with 12 occurrences. Subsequently, the presence of *MCR*-1 and *MCR*-8 was detected in 10 and 8 isolates, respectively. **Conclusion:** This study represents the inaugural report on colistin resistance in Gram-negative bacteria obtained from poultry farms in the West Bank region of Palestine. The results of our study serve as a catalyst for raising awareness about the emergence of colistin resistance in animal farms, particularly in poultry, and the significant implications this has for human health.

Keywords: Colistin Resistance, Multi-Drug Resistant, Bacterial Resistance, Bactria Poultry Farm.

INTRODUCTION

Antibiotic resistance among Gram-negative bacteria first gained attention in the 1970s, and since then, the prevalence of antibiotic resistance in these bacteria has been steadily increasing worldwide [1]. This rise in resistance has reached alarming levels among many bacterial pathogens, leading to a global crisis. It poses a significant threat to human and veterinary medicines, jeopardizing the effectiveness of commonly used antibiotics. In addition, this situation poses a serious risk to food safety and human health worldwide. [2, 3].

Polymyxin is a class of antibiotics consisting of five members: polymyxin A, B, C, D, and E. However, only polymyxin B and E (colistin) have been approved for clinical use [4-6]. Colistin is produced by the gram-positive bacterium *Paenibacillus polymyxa subsp. colistinus* was discovered in 1947 and was used topically in Europe and Japan in the 1950s [1]. Its clinical application was initially limited to topical use due to its nephrotoxic and neurotoxic properties [7].

Colistin is an antibiotic with a narrow spectrum that targets Gram-negative bacteria, specifically those belonging to the *Enterobacteriaceae* family [8]. It disrupts these bacteria's outer membrane by interacting with the lipopolysaccharide (LPS) phosphate groups, stabilizing the membrane [9]. Colistin displaces divalent cations from the phosphate groups, causing disorganization and destabilization of the outer membrane [4]. This leads to the leakage of cellular compounds and ultimately results in the death of the bacterial cells [6].

In recent years, colistin has been reintroduced as a last choice for the treatment of infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria [1, 6, 10]. In addition, the World Health Organization (WHO) included colistin in the "Highest Priority Important Antimicrobials" group for human medicine because of its high impact [11]. Interestingly, colistin is a common therapeutic agent in veterinary medicine. It is used in animal agriculture as a prophylactic antibiotic and promoter for animal growth and dairy production [5, 8, 12, 13].

The misuse and overuse of colistin in livestock and poultry farms have developed colistin resistance, turning these farms into reservoirs for resistant bacteria [12, 14]. Initially, colistin resistance in *Enterobacteriaceae* was attributed to mutations in specific genes (*pmrA/B, phoP/Q*, and *mgrB*) that regulate the production of enzymes involved in modifying lipopolysaccharide (LPS) [15, 16]. Moreover, these farms contribute to the spread of colistin resistance genes among different bacterial species [17, 18].

This covalent modification of LPS involves incorporating positively charged groups that neutralize LPS negative charges, thus reducing the binding of cationic colistin to its target [2]. The colistin resistance mentioned above was considered the most common among the family Enterobacteriaceae bacterial species [2, 19]. Other alternative mechanisms that have been described to mediate colistin resistance involve the overexpression of an efflux pump system and the overproduction of capsular polysaccharides [19].

Currently, the acquisition of plasmid-mediated genes, known as mobile colistin resistance (*MCR*) genes [6], is responsible for most of the cases of colistin resistance seen among *E. coli*, *Salmonella*, and some *K. pneumoniae* isolates [20]. Ten *MCR* gene variants (*MCR*-1 to *MCR*-10) have been described [21]. Each of these genes encodes a phosphoethanolamine (pEtN) transferase enzyme that modifies the lipid-A component of LPS to interfere with colistin binding to its target [22].

The *MCR*-1 gene was initially reported in *E. coli* isolates obtained from pigs and meat in China [1]. However, global attention was not directed to colistin resistance until the discovery of the *MCR*-1 gene in *E. coli* isolates obtained from animal food [23]. Currently, the *MCR*-1 gene and, to a lesser extent, the *MCR*-3 gene are the most prevalent *MCR* genes worldwide [8, 24].

This study aims to fill the knowledge gap by examining the prevalence of colistin resistance mediated by the *MCR*-1 gene and other *MCR* gene variants in Gram-negative *Enterobacteriaceae* isolates obtained from poultry farms in Palestine. As far as we know, no previous research has been conducted in the West Bank.

MATERIAL AND METHODS

Bacterial Isolate Sources

A total of ninety cloacal swabs were collected from thirty poultry farms, and three cloacal swabs were collected from three individual hens from each farm, distributed all over Nablus governorate, West Bank, from March to June 2019. A veterinarian collected swabs with transport media under the supervision of the director of the Veterinary Directorate in Nablus Governorate. During the study period, only occupied farms were targeted and visited once, covering all regions in the governorate under the guidance of the director. All swabs were collected aseptically using sterile swabs to avoid contamination by a veterinarian under the guidance and monitoring of a microbiologist.

Isolation and identification of the Gram-negative bacteria

Each cloacal swab was streaked on both MacConkey agar (Mac) (Himedia, India) and Eosin Methylene Blue agar (EMB) plates (Himedia, India). The plates were incubated at 35±2°C for 24-36h. After incubation, three different colonies were picked and re-streaked on Mac agar to obtain pure cultures. The plates were incubated again at 35±2°C for 24-36h. According to the manufacturer's instructions, the Oxidase test (ROTH, Germany) and the API-20E (bioMerieux, Inc., France) identified lactose fermenter and non-fermenter isolates.

Antimicrobial Susceptibility Testing

The disc diffusion method was employed to conduct antimicrobial susceptibility testing on each bacterial isolate, following the criteria and methods outlined by the Clinical Laboratory Sciences Institute (CLSI, 2019) [25]. We employed antimicrobial discs manufactured commercially by Liofilchem, Italy, in this investigation. The first two are trimethoprim and tetracycline and then comes trimethoprim-sulfamethoxazole, ciprofloxacin, chloramphenicol, ampicillin, gentamicin, fosfomycin, cefuroxime, cefotaxime, ceftriaxone, cefzil, amikacin, imipenem, and meropenem. Muller-Hinton (MH) agar plates (obtained from Himedia, India) were prepared by placing discs on their swabbed surface. The test organism inoculum was adjusted to a 0.5 McFarland standard before being spread across the agar plates. Antimicrobials were allowed to diffuse properly into the plates after being chilled for 15 minutes. The plates were then incubated for 24-36 hours at a temperature of 35 °C. CLSI 2019 guidelines and tables supplied by the antimicrobial disc manufacturer were used to interpret the data.

Colistin Minimum Inhibitory Concentration (MIC)

The determination of colistin MIC values against the isolated bacteria was conducted using the micro broth dilution method, as previously mentioned [26, 27]. In other words, the 96-microtiter plate was used to perform a two-fold serial dilution of colistin obtained in pure form from Sigma Aldrich Inc. The dilution range spanned from 64 - 0.5 mg/l. Bacterial inoculum was standardized in each well. Following an incubation period of 16-20 hours, 20 microliters of tetrazolium chloride were introduced into each well and incubated for 15 minutes. The MIC was determined by assessing the intensity of color development. Without breakpoint guidelines for *Enterobacteriaceae* in colistin testing from the CLSI, we relied on the colistin MIC breakpoints provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for interpretation. According to EUCAST, isolates with MIC values of ≤2 mg/l are considered susceptible, while those with MIC values beyond 2 mg/l are classified as resistant [28].

PCR amplification of 8 MCR gene variants

The eight *MCR* gene variants (*MCR*-1 to *MCR*-8) were analyzed as previously reported [21, 24]. A conventional PCR was performed for the eight *MCR* gene variants (*MCR*-1 to *MCR*-8). PCR reactions were conducted on a final volume of 25 µl of the reaction mixture (2 µl of the DNA template, 1 µl of each forward and reverse primer, 5 µl of Master Mix

(Thermoscientific, USA), and 16 µl of nuclease-free water). The DNA amplification was performed in a Thermal cycler (Bio-Rad, USA). The initial denaturation occurred at 94 °C for 15 minutes, then 25 cycles were conducted as follows: 1- Denaturation: 94 °C for 30 seconds. 2- Annealing: 58 °C for 90 seconds. 3- Extension: 72 °C for 60 seconds. The final extension was done at 72 °C for 10 minutes. The PCR products were detected by 2 % agarose gel electrophoresis in Tris/Borate/EDTA (TBE) buffer. Gels were stained with 10 µl ethidium bromide, and the amplified bands were visualized by the Gel Documentation System (UVP, PRC) under a UV illuminator.

RESULTS

Isolation of Gram-negative bacteria

From the sampled cloacal swabs of the thirty poultry farms, 244 Gram-negative isolates were obtained. The commonly isolated bacteria belonged to *Enterobacteriaceae* 232/244 (95.1%), including *E. coli* 103/244 (42.2%), followed by *Proteus mirabilis* and *Salmonella* spp. 24/244 (9.8%) and 19/244 (7.8%), respectively. Interestingly, 62/244 (25.4 %) isolated Gram-negative bacteria were *Serratia* spp. (Figure 1).

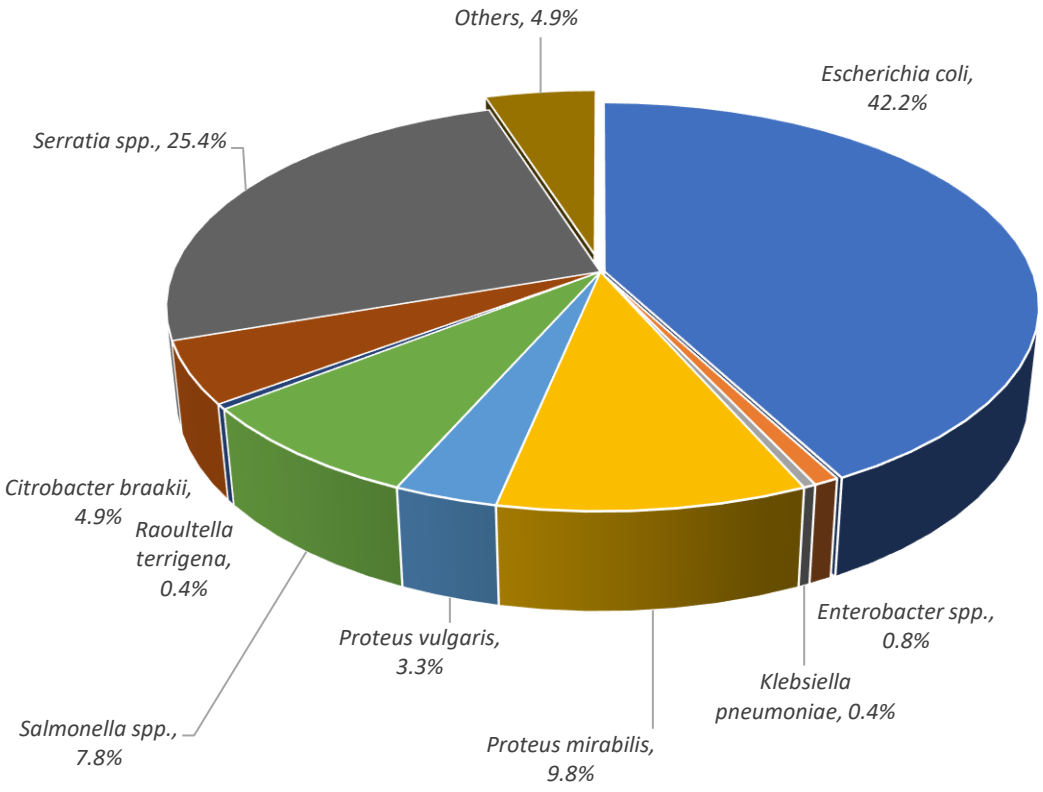

Figure (1): Distribution of Gram-negative isolates from poultry farms.

Antimicrobial resistance profile of Gram-negative bacterial isolates.

Varying degrees of susceptibilities were observed for the isolates against the tested antimicrobial agents, even within members of the same antimicrobial class. Imipenem was the most effective drug, with a resistance of 0.0%, followed by meropenem 1/244 (0.4%), amikacin 3/244 (1.2%), and ceftazidime 5/244 (2%). At the same time, high resistance was recorded against trimethoprim 244/244 (100%) and tetracycline 230/244 (94.3%). Surprisingly, a high resistance rate was shown against colistin 155/237 (65.4%) (Table 1). A detailed antimicrobial profile for each Gram-negative bacterium is shown in the supplementary materials (Table S1). Moreover, among the *Enterobacteriaceae* isolated in this study, high rates of MDR 140/167 (83.8%), XDR 8/167 (4.8%), and ESBL 31/167 (18.6%) were detected (Table S2).

Table (1): Antimicrobial resistance profile for 244-gram negative isolates.

Antimicrobials	Resistance		Intermediate		Susceptible		total
	#	%	#	%	#	%	
Amikacin	3	1.2	5	2.0	236	96.7	244
Ampicillin	184	75.4	7	2.9	53	21.7	244
Ceftazidime	5	2.0	5	2.0	234	95.9	244
Cefotaxime	25	10.2	3	1.2	216	88.5	244
Ceftriaxone	17	7.0	8	3.3	219	89.8	244
Cefuroxime	25	10.2	16	6.6	203	83.2	244
Chloramphenicol	197	80.7	7	2.9	40	16.4	244
Ciprofloxacin	210	86.1	18	7.4	16	6.6	244
Fosfomycin	42	17.2	5	2.0	197	80.7	244
Gentamicin	123	50.4	26	10.7	95	38.9	244
Imipenem	0	0.0	14	5.7	230	94.3	244
Meropenem	1	0.4	0	0.0	243	99.6	244
Tetracycline	230	94.3	1	0.4	13	5.3	244
Trimethoprim	244	100.0	0	0.0	0	0.0	244
TM/SXT *	221	90.6	3	1.2	20	8.2	244
Colistin**	155	65.4	(7 missed samples)		82	34.6	237

** Trimethoprim-Sulfamethoxazole

*Colistin was tested using micro-broth dilution, while all other antibiotics were tested using disk diffusion.

Table (S1): Antibiotic resistance profile for each isolated bacterium.

Bacteria name Antobiotics		*Enterobacteriaceae* (170)																Others (74)								
		E. coli (102)		*Entero-bacter spp.* (2)		*k. pneu-mu-nia* (1)		*P. mi-rabi-lis* (24)		*P.vul garis* (8)		*Salmo-nella spp.* (19)		*R. ter-rigene* (1)		*C. bra akii* (12)		*A.s hy-droph ila* (1)		*Ser-ratia spp.* (62)		Othe r non-iden-tified (11)	
			%		%		%		%		%		%		%		%		%		%		%
Ampicillin AMP	S	13	12.6	0	0	1	100	18	75	6	75	1	5.3	0	0	6	50.0	0	0	9	14.5	3	27.3
	I	3	2.9	0	0	0	0	0	0	0	0	0	0.0	1	100	0	0.0	1	100	2	3.2	0	0.0
	R	87	84.5	2	100	0	0	6	25	2	25	18	94.7	0	0	6	50.0	0	0	51	82.3	8	72.7
Imipenem IPM	S	98	95.1	2	100	1	100	24	100	6	75	17	89.5	1	100	8	66.7	1	100	61	98.4	11	100.0
	I	5	4.9	0	0	0	0	0	0.0	2	25	2	10.5	0	0	4	33.3	0	0	1	1.6	0	0.0
	R	0	0.0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
Cefotaxime CTX	S	87	84.5	1	50	1	100	22	91.7	8	100	15	78.9	1	100	11	91.7	1	100	59	95.2	10	90.9
	I	1	1.0	0	0	0	0	0	0.0	0	0	1	5.3	0	0	0	0.0	0	0	1	1.6	0	0.0
	R	15	14.6	1	50	0	0	2	8.3	0	0	3	15.8	0	0	1	8.3	0	0	2	3.2	1	9.1
Tetracycline TE	S	3	2.9	0	0	1	100	3	12.5	1	12.5	0	0.0	1	100	1	8.3	0	0	3	4.8	0	0.0
	I	0	0.0	0	0	0	0	0	0.0	1	12.5	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
	R	100	97.1	2	100	0	0	21	87.5	6	75	19	100.0	0	0	11	91.7	1	100	59	95.2	11	100.0
Ciprofloxacin CIP	S	3	2.9	0	0	1	100	3	12.5	3	37.5	1	5.3	0	0	2	16.7	0	0	1	1.6	3	27.3
	I	4	3.9	0	0	0	0	7	29.2	4	50	1	5.3	0	0	0	0.0	0	0	2	3.2	0	0.0
	R	96	93.2	2	100	1	100	14	58.3	1	12.5	17	89.5	1	100	10	83.3	1	100	59	95.2	8	72.7
Meropenem MEM	S	103	100.0	2	100	1	100	24	100.0	7	87.5	19	100.0	1	100	12	100.0	1	100	62	100.0	11	100.0
	I	0	0.0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
	R	0	0.0	0	0	0	0	0	0.0	1	12.5	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
Fosfomycin FF	S	80	77.7	0	0	1	100	21	87.5	5	62.5	17	89.5	1	100	9	75.0	1	100	54	87.1	8	72.7
	I	2	1.9	0	0	0	0	1	4.2	0	0	0	0.0	0	0	1	8.3	0	0	1	1.6	0	0.0
	R	21	20.4	2	100	0	0	2	8.3	3	37.5	2	10.5	0	0	2	16.7	0	0	7	11.3	3	27.3
Gentamicin CN	S	34	33.0	1	50	1	100	9	37.5	3	37.5	9	47.4	1	100	7	58.3	0	0	27	43.5	3	27.3
	I	15	14.6	0	0	0	0	2	8.3	1	12.5	0	0.0	0	0	1	8.3	0	0	6	9.7	1	9.1
	R	54	52.4	1	50	0	0	13	54.2	4	50	10	52.6	0	0	4	33.3	1	100	29	46.8	7	63.6

Bacteria name Antobiotics		*Enterobacteriaceae* (170)																Others (74)					
		E. coli (102)	%	*Entero-bacter spp.* (2)	%	*k. pneu-mu-nia* (1)	%	*P. mi-rabi-lis* (24)	%	*P.vul garis* (8)	%	*Salmo-nella spp.* (19)	%	*R. ter-rigene* (1)	%	*C. bra akii* (12)	%	*A.s hy-droph ila* (1)	%	*Ser-ratia spp.* (62)	%	Othe r non-iden-tified (11)	%
Cefazidime CAZ	S	98	95.1	1	50	1	100	24	100.0	7	87.5	18	94.7	1	100	12	100.0	1	100	61	98.4	10	90.9
	I	3	2.9	0	0	0	0	0	0.0	1	12.5	0	0.0	0	0	0	0.0	0	0	1	1.6	0	0.0
	R	2	1.9	1	50	0	0	0	0.0	0	0	1	5.3	0	0	0	0.0	0	0	0	0.0	1	9.1
Trime-thoprim-Sul-phamethoxa-zole SXT	S	9	8.7	0	0	1	100	2	8.3	0	0	2	10.5	1	100	0	0.0	0	0	3	4.8	2	18.2
	I	0	0.0	0	0	0	0	1	4.2	0	0	0	0.0	0	0	1	8.3	0	0	1	1.6	0	0.0
	R	94	91.3	2	100	0	0	21	87.5	8	100	17	89.5	0	0	11	91.7	1	100	58	93.5	11	100.0
trimethoprim W	S	0	0.0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
	I	0	0.0	0	0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0	0	0.0	0	0.0
	R	103	100.0	1	50	1	100	24	100.0	8	100	19	100.0	1	100	12	100.0	1	100	6	9.7	11	100.0
Chloram-phenicol C	S	14	13.6	0	0	0	0	3	12.5	3	37.5	3	15.8	0	0	4	33.3	0	0	11	17.7	2	18.2
	I	3	2.9	0	0	0	0	1	4.2	0	0	1	5.3	0	0	0	0.0	0	0	2	3.2	0	0.0
	R	86	83.5	2	100	1	100	20	83.3	5	62.5	15	78.9	1	100	8	66.7	1	100	49	79.0	9	81.8
Amikacin AK	S	100	97.1	2	100	0	0	22	91.7	8	100	17	89.5	1	100	11	91.7	1	100	62	100.0	11	100.0
	I	2	1.9	0	0	1	100	1	4.2	0	0	1	5.3	0	0	0	0.0	0	0	0	0.0	0	0.0
	R	1	1.0	0	0	0	0	0	0.0	0	0	1	5.3	0	0	1	8.3	0	0	0	0.0	0	0.0
Ceftriaxone CRO	S	89	86.4	2	100	1	100	22	91.7	8	100	16	84.2	1	100	11	91.7	1	100	58	93.5	10	90.9
	I	2	1.9	0	0	0	0	1	4.2	0	0	3	15.8	0	0	0	0.0	0	0	2	3.2	0	0.0
	R	12	11.7	0	0	0	0	1	4.2	0	0	0	0.0	0	0	1	8.3	0	0	2	3.2	1	9.1
Cefuroxime CXM	S	81	78.6	1	50	1	100	22	91.7	8	100	16	84.2	1	100	9	75.0	1	100	55	88.7	8	72.7
	I	9	8.7	1	50	0	0	0	0.0	0	0	1	5.3	0	0	1	8.3	0	0	3	4.8	1	9.1
	R	13	12.6	0	0	0	0	2	8.3	0	0	2	10.5	0	0	2	16.7	0	0	4	6.5	2	18.2
*Colistin	S	43	42.2	2	100	0	0	1	4.4	0	0	9	47.4	1	100	0	0.0	0	0	21	34.4	5	62.5
	R	59	57.8	0	0	1	100	22	95.6	7	100	10	52.6	0	0	12	100.0	1	100	40	65.6	3	37.5

*Colistin tested using micro-broth dilution, while all other antibiotics were tested using disk diffusion.

Table (S2): MDR, XDR, and ESBL percentage among the isolated Gram-negative bacteria.

Bacterial species (No.)	MDR (%)	XDR (%)	ESBL (%)
***Enterobacteriaceae* (167)**	84	5	19
Escherichia coli (102)	75	4	15
Enterobacter spp. (2)	100	0	50
Klebsiella pneumunia (1)	100	0	0
Proteus mirabilis (23)	60	4	9
Proteus vulgaris (7)	86	14	43
Salmonella spp. (19)	74	11	21
Raoultella terrigene (1)	100	0	0
Citrobacter braakii (12)	75	8	17
Others (70)	89	2	8
Aeromonas hydrophila (1)	100	0	0
Serratia spp. (61)	80	5	23
Other non-identified (8)	88	0	0
MDR = Multi-Drug Resistant (resistant to at least one agent in three or more antimicrobial classes)			
XDR = Extensively drug-resistant (Susceptible to two or fewer antimicrobial classes)			
ESBL = Extended-spectrum β- lactamases			

Colistin MIC

Bacterial isolates that showed resistance at a concentration higher than 2 mg/l were classified as resistant. Only 82/237 (34.6%) of the tested isolates were considered susceptible, while 155/237 (65.4%) were classified as resistant. Table 2 provides the MIC values obtained for 237 Gram-negative isolates tested against colistin sulfate. The highest colistin resistance was found among *Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis, Citrobacter braakii, Aeromonas hydrophila,* and *Serratia spp* and followed by *E. coli* 59/102 (57.8%) (Table 2).

Table (2): Colistin-resistant concentration (mg/l) for isolated bacteria, using Microbroth dilation.

Identified bacteria (number)	Resistant										Susceptible							
	> 16	%	16	%	8	%	4	%	Total	%	2	%	1	%	0.5	%	Total	%
Enterobacteriaceae (167)	98	58.7	2	1.2	5	3.0	6	3.6	111	66.5	23	13.7	24	14.4	9	5.4	56	33.5
Escherichia coli (102)	49	48.0	1	1.0	3	2.9	6	5.9	59	57.8	19	18.6	17	16.7	7	6.9	43	42.2
Enterobacter spp. (2)	-	-	-	-	-	-	-	-	0	0.0	-	-	1	50	1	50	2	100.0
klebsiella pneumunia (1)	-	-	-	-	1	100	-	-	1	100.0	-	-	-	-	-	-	0	0.0
Proteus mirabilis (23)	22	95.7	-	-	-	-	-	-	22	95.7	1	4.3	-	-	-	-	1	4.3
Proteus vulgaris (7)	7	100	-	-	-	-	-	-	7	100.0	-	-	-	-	-	-	0	0.0
Salmonella spp. (19)	8	42.1	1	5.3	1	5.3	0	0.0	10	52.7	2	10.5	6	31.6	1	5.3	9	47.3
Raoultella terrigene (1)	-	-	-	-	-	-	-	-	0	0	1	100	-	-	-	-	1	100
Citrobacter braakii (12)	12	100	-	-	-	-	-	-	12	100.0	-	-	-	-	-	-	0	0.0
Others (70)	31	44.3	7	10.0	2	2.9	4	5.7	44	62.9	6	8.6	11	15.7	9	12.8	26	37.1
Aeromonas hydrophila (1)	-	-	-	-	-	-	1	100	1	100.0	-	-	-	-	-	-	0	0.0
Serratia spp. (61)	28	45.9	7	11.5	2	3.3	3	4.9	40	65.6	6	9.8	8	13.1	7	11.5	21	24.6
Other non-identified (8)	3	37.5	-	-	-	-	-	-	3	37.5	-	-	3	37.5	2	25	5	62.5
									155	65.4							82	34.6

3.4 PCR amplification of MCR gene variants

Eight *MCR* gene variants were targeted in this study, representing members of the plasmid-mediated genes responsible for colistin resistance among *Enterobacteriaceae*. Table 3 shows the type of *MCR* genes among those *Enterobacteriaceae* isolates considered resistant to colistin according to the MIC results. *MCR* gene variants were detected in 35/111 isolates (31.5%). The most frequent gene variant among *Enterobacteriaceae* isolates is *MCR-7*, detected in twelve isolates. Then, *MCR-1* and *MCR-8* were found in ten and eight isolates, respectively.

On the other hand, *MCR-3* and *MCR-4* were not found in our isolates. Regarding *E. coli*, among those isolates that were resistant to colistin, *MCR* gene variants were detected in 13/59 isolates (22%). The most frequent gene variant is *MCR*-1, detected in five isolates.

Table (3): *MCR* gene variants among colistin-resistant *Enterobacteriaceae* isolates.

	***MCR* gene variants**								**Negative**	**Total**
	MCR-1	***MCR-2***	***MCR-3***	***MCR-4***	***MCR-5***	***MCR-6***	***MCR-7***	***MCR-8***		
No. of *Enterobacteriaceae* isolates	10	2	0	0	1	2	12	8	99	134
No. of *E. coli* isolates	5	1	0	0	0	2	3	2	65	78

DISCUSSION

This study represents the inaugural investigation documenting the assessment of antimicrobial susceptibility, with a particular focus on colistin resistance, in Gram-negative bacteria obtained from poultry farms in the West Bank region of Palestine. The predominant bacteria isolated from the sampled swabs were found to be from the Enterobacteriaceae family, accounting for 69.7% (170 out of 244) of the samples. Among these, Escherichia coli was the most commonly identified bacterium in 42.2% (103 out of 244) of the samples. Other isolated bacteria included Proteus mirabilis and Salmonella spp., found in the samples in 9.8% (24 out of 244) and 7.8% (19 out of 244), respectively. Notably, a significant proportion of the Gram-negative bacteria, specifically 62 out of 244 (25.4%), comprised isolated Serratia spp. Diverse levels of susceptibility toward the antimicrobials tested were noted among the isolates, including variations within members of the same antimicrobial category. Meropenem demonstrated the highest efficacy among the drugs assessed, achieving a success rate of 243 out of 244 cases (99.6%). Subsequently, amikacin exhibited a success rate of 236 out of 244 cases (96.7%). Trimethoprim exhibited a high resistance rate of 100% (244/244), while tetracycline demonstrated a resistance rate of 94.3% (230/244). Remarkably, a study conducted in the Gaza Strip revealed a considerable resistance rate of 65.4% (155 out of 237) against colistin, consistent with findings from a previous investigation on Escherichia coli isolated from chicken feces [29].

In this study, the rate of colistin resistance was 65.4% among Gram-negative isolates, which is slightly higher than the rate reported (63.4%) in a study conducted on strains collected from a range of countries [30]. Moreover, our results show a high rate of colistin resistance among *E. coli* isolates (N=59; 57.8%), higher than the rate of colistin resistance in the study conducted in the Gaza Strip [29]. We are reporting a higher rate of colistin resistance compared to the published data in Kuwait in 2018 by Alfoiuzan et al., where the team reported resistance of 4.3% for *E. coli* [31]. Variations in colistin usage may explain this variation in resistance rates between various studies, the source of isolates tested, and the study period.

Our finding triggers an alert on colistin resistance in animal farms, mainly poultry, and its detrimental effects on human health. The detection of a high prevalence of colistin

resistance among Gram-negative bacteria might correlate with the massive use of antibiotics in poultry farms as growth enhancers and as prophylaxis. Aris *et al.* studied the prevalence of colistin resistance in *K. pneumoniae* in the Middle East. They mentioned that there has been no report of colistin resistance among *K. pneumoniae* isolates in Palestine [32]. Recently, Qadi *et al.* reported that 31.6% of *K. pneumoniae* strains isolated from clinical samples from the Gaza Strip were colistin-resistant [26]. Our isolates in this study recovered from poultry farms, and only one *K. pneumoniae* was isolated; nevertheless, this isolate was resistant to colistin.

Carbapenem-resistant Gram-negative pathogens have become a major healthcare burden in the 21st century, and treatment options have been limited to agents such as colistin and tigecycline in combination with other antibiotics [33]. In this study, only one bacterial isolate of meropenem-resistant *Enterobacteriaceae* was resistant to colistin, while fourteen isolates of imipenem-intermediate *Enterobacteriaceae* were resistant to colistin (data not shown). Our findings highlight how much resistance to colistin has increased within the last decade. For example, ten years ago in England, the activity of colistin was evaluated against 81 carbapenem-resistant *Enterobacteriaceae* isolates, and colistin was active against 92.6% of the isolates [34]. In a study of colistin resistance in *K. pneumoniae* and *E. coli* strains isolated from cancer patients, 45% of colistin-resistant isolates were meropenem-resistant [35]. In another study, the imipenem and meropenem resistance rates in Gram-negative rods were 8.1% and 0.8%, respectively [36]. In another study, the rate of imipenem resistance in Extended Spectrum Beta Lactamase (ESBL)-producing isolates of *Enterobacteriaceae* was 20% [37]. Those reported rates are higher than the rate determined by our study, which was less than 1%. This assertion is highly justifiable, given that the research was conducted on bacterial strains obtained from poultry farms rather than clinical environments. The findings of this study are indeed concerning, emphasizing the need for heightened scrutiny regarding the utilization of antibiotics within animal farming operations, particularly those that are economically accessible.

Among those strains resistant to colistin, *MCR-7,* followed by *MCR-1,* were the most prevalent *MCR* genes. The *MCR-7* gene was first detected and described in 2018 in *K. pneumoniae* isolated from chicken [38]. Shi *et al.* showed a high prevalence and rate of carbapenem and colistin resistance in livestock farm environments in China. They showed that the colistin resistance gene *MCR-1* threatened food safety and public health and was selected to investigate antibiotic resistance gene pollution in farm environments [39]. Das *et al.* showed that 58% of commensal *E. coli* were resistant to colistin and had *MCR-1*-resistant plasmid [40]. Recently, Al-Mir *et al.* analyzed the Whole Genome Sequencing (WGS) of clonal and plasmid epidemiology of colistin resistance mediated by *MCR* genes in the poultry Sector in Lebanon. They showed 27/32 (84.4%) *MCR-1* positive farms, leading to 84 non-duplicate E. coli collected, of which 62% harbored the *MCR-1* gene [41]. Another study from Lebanon showed the occurrence of the colistin resistance gene *MCR-1* and additional antibiotic resistance genes in ESBL/AmpC-producing *E. coli* from poultry. They showed the genotype distribution of colistin-resistant *E. coli* across Lebanon [42]. According to other studies, *MCR-3* is the second most prevalent *MCR* gene worldwide [24, 43]. In our study, no isolates harbored the *MCR-3* gene. This finding highlights the importance of studying all *MCR* gene variants.

This study's findings highlight the need to implement measures aimed at mitigating the inappropriate use and excessive administration of antibiotics, including carbapenems and colistin. This is particularly crucial in light of the observed elevated prevalence of MDR strains. Finally, our study calls upon policymakers to spread awareness among farmers and the public in Palestine to stop and control the massive use of antibiotics in animal farms, especially those used for humans. Also, laws and regulations that regulate the use of antibiotics in animal farms should be passed. Furthermore, it is vital to set up screening programs to routinely test for antibiotics in farms, animal meat, and products in the market. Finally, further studies should be conducted to understand the molecular basis behind colistin resistance in Palestine.

CONCLUSION

In summary, the present study conducted in the West Bank region of Palestine aimed to investigate antimicrobial resistance patterns, specifically colistin resistance, within Gram-negative bacteria obtained from poultry farms. The study's results indicated the presence of elevated levels of resistance, encompassing both multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. The research revealed a notable prevalence of colistin resistance within the Enterobacteriaceae group, with Escherichia coli being the predominant species. The prevalence of colistin resistance in our research study can be attributed to the selective pressure exerted by the extensive utilization of colistin as a vital antibiotic in clinical settings, primarily employed as a final option for treating infections resistant to multiple drugs. The resistant isolates contained various *MCR* gene variants, including but not limited to *MCR*-1, *MCR*-7, and *MCR*-8. The mobile capacity of plasmids harboring specific *MCR* variants is associated with the occurrence of these variants within particular populations. The study underscores the pressing necessity for implementing surveillance and control strategies to tackle antimicrobial resistance in poultry farms, drawing attention to the potential hazards to human health. Implementing judicious antibiotic utilization, antimicrobial stewardship initiatives, and improved surveillance systems is imperative in addressing the proliferation of multidrug-resistant microorganisms and safeguarding the health and welfare of animals and humans.

Ethics approval and consent to participate

Ethical approval was obtained from the Institutional Review Board (IRB) at An-Najah National University in Palestine. Additional approval was obtained from the Palestinian Ministry of Agriculture to access poultry farms.

Consent for publication

All authors read and approved the final manuscript.

Data Availability

The data used to support the study's findings is in the paper and the supplementary file.

Author's contribution

Mohammad Qadi: Conceptualization, writing-original draft, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization and editing. **Rasha Khayyat:** Conceptualization, formal analysis, funding acquisition, investigation, project administration, resources. **Safaa Alhato:** Data curation, formal analysis, methodology, visualization and editing. **Abdelraouf Elmanama:** Conceptualization, formal analysis, funding acquisition, investigation, methodology, visualization and editing. **Ashraf Zayed and Muna Abbas:** Writing-original draft, visualization and editing. **Maysa Daqqa, Faizeh Hussein and Ahmed Mousa:** Investigation and Methodology.

Conflicts of Interest

The authors certify that there are no competing interests.

FUNDING

This research was funded by Al Maqdisi Program 2018-2020 (French Consulate Jerusalem).

ACKNOWLEDGMENTS

The author(s) would like to thank An-Najah National University (www.najah.edu) for the technical support in publishing the present work. The authors are also thankful for the Palestinian Ministry of Agriculture for facilitating this work

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