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Colistin Resistant *Escherichia coli* Isolated from Chicken Fecal Materials in Gaza Strip

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Abstract:

Background: Colistin is considered as last line in treating infections caused by carbapenem-resistant *Enterobacteriacae* (CRE). Resistance of *E. coli* against colistin comprise a major public health threat. Uncontrolled use of colistin and other antimicrobials in the poultry industry in Gaza strip probably accelerated the emergence of resistance to colistin and the spread to human.

Objective: The main objective of this study is to determine the occurrence of colistin resistance in isolates of *E. coli* from chicken fecal materials in Gaza strip, Palestine

Methods: One hundred and ten chicken litter samples were collected from different poultry farms (Gaza and middle governorates). All samples were cultured onto Eosin Methylene Blue and MacConkey Agar plates. *E. coli* were identified biochemically. Disk diffusion method was used to determine the antimicrobial susceptibility for 11 antimicrobial agents (Ceftriaxone, Chloramphenicol, Ampicillin, Co-trimazol, Meropenem, Amikacin, Norfloxacin, Nalidixic acid, Neomycin, Cephalothin, and Colistin).

Result: There were wide variations in the susceptibilities of the different *E. coli* isolates to these antimicrobials. Resistance to Ampicillin was detected in all 110 (100%) isolates, followed by Cephalothin (86.4%), Cotrimazol (67.3%), Nalidixic acid (52.7%), Chloramphenicol (43.6%), Ceftriaxone (30.9%), Neomycin (17.3%), Norfloxacin (15.5), Colistin in

16 (14.5%), Meropenem in 5 (4.5%), and Amikacin (0.9%). Of the tested isolates, 76.4% (84) were multidrug resistant (MDR).

Conclusion: Phenotypic resistance to colistin sulfate was found in 16 (14.5%) isolates. Resistance to meropenem was found in 5 (4.5%) isolates from the total of 110 isolates and 76.4% (84) were MDR. This requires immediate action by the concerned authorities to regulate and monitor the use of antimicrobials in the poultry industry.

Keywords: Colistin resistance, *E. coli*, Gaza, Palestine

ملخص عربي

الخلفية: يعتبر المضاد الحيوي الكوليستين الخيار الأخير في علاج العدوى التي تسببها البكتيريا سالبة الجرام، وخاصة البكتيريا المعوية المقاومة للكاربابنيمات (CRE). تشكل مقاومة الإشريكية القولونية (*E. coli*) للكولستين مصدر تهديد رئيسي للصحة العامة. ومن المحتمل أن يساهم الاستخدام غير المنظم للكولستين وغيره من مضادات الميكروبات في تربية الدواجن في قطاع غزة في تسريع ظهور المقاومة ضد الكوليستين وانتقالها للبشر.

الهدف: الهدف الرئيسي من هذه الدراسة هو تحديد مدى حدوث مقاومة الكوليستين في عزلات الإشريكية القولونية (*E. coli*) من براز الدجاج في قطاع غزة، فلسطين

الطريقة: تم جمع 110 عينه من أماكن مختلفة (غزة والمحافظات الوسطى). تمت زراعتهم على اوساط زراعية مختلفة مثل (Eosin Methylene Blue و MacConkey Agar). تم التعرف على الإشريكية القولونية (*E. coli*) كيميائيا. ثم تم استخدام طريقة انتشار القرص لتحديد حساسية البكتيريا لـ 11 من المضادات الحيوية (Cetriaxone، Chloramphenicol، Ampicillin، Co-trimazol، Meropenem، Amikacin، Norfloxacin، Nalidixic acid، Neomycin، Cephalothin، Colistin).

النتائج: كانت هناك اختلافات واسعة في حساسية عزلات *E. coli* للمضادات الميكروبية التي تم استخدامها. كانت مقاومة Ampicillin في جميع العزلات (100 ٪)، يليها Cephalothin (86.4 ٪)، Cotrimazol (67.3 ٪)، Nalidixic acid (52.7٪)، Chloramphenicol (43.6%) ، Ceftriaxone (30.9%) ٪(، Neomycin (17.3% ، Norfloxacin (15.5) ، Colistin في 16 عينة (14.5 ٪) ، Meropenem في عينات 5 (4.5 ٪)، و Amikacin (0.9%) من

جميع العزلات التي تم اختبارها ، كانت نسبة المقاومة المتعددة المضادات الميكروبية (Multidrug Resistant (MDR)) 76.4 ٪ ل (84) عينه.

الاستنتاجات: المقاومة للكولستين تم تحديدها في 16 عزلة (14.5 ٪). تم العثور على مقاومة ضد Meropenem في 5 (4.5 ٪) عزلة من مجموع 110 عزلة. كانت نسبة المقاومة المتعددة للمضادات (MDR) 76.4 ٪ (84). وهذا يتطلب إجراءات فورية من قبل السلطات المعنية لتنظيم ومراقبة استخدام مضادات الميكروبات في تربية الدواجن.

الكلمات المفتاحية: مقاومة الكوليستين، الإشريكية القولونية، غزة –فلسطين

Background

Escherichia coli is considered as the normal bowel flora of different species of mammals and birds but some strains of *E. coli* possess pathogenic character due to the acquisition of virulent factors. Microbial characteristics associated with virulent *E. coli* include production of enterotoxin, verotoxin, colicins and siderophores, type-1 pili and motility, resistance to the lytic action of the host complement and antibiotics (Dho and Lafont 1984).

E. coli is one of the most important etiological agents causing diseases in poultry which leads to significant economic losses related to high mortality, poor weight gain of infected chicken and poor carcass quality (Ewers, Janßen et al. 2004). Colistin (Polymyxin E) is an antibiotic produced by certain strains of *Paenibacillus polymyxa*. It is a mixture of the cyclic polypeptides colistin A and B. Colistin was first isolated in Japan from *Bacillus polymyxa* var. colistinus by the Japanese scientist Koyama in 1949 and became available for clinical use in 1959. Colistin is effective against most of the Gram negative bacilli (Kempf, Fleury et al. 2013).

Colistin resistance is a gradually emerging problem among gram-negative bacteria in clinical settings in many countries (Olaitan, Diene et al. 2014). Colistin is the last line of defense for carbapenem-resistant Enterobacteriaceae (CRE) or *Acinetobacter baumannii* infection (Li, Nation et al. 2006). It is a drug once considered to be inconvenient and too toxic for routine parenteral use, into daily clinical application (Dhariwal and Tullu 2013). As for these pathogens colistin often represents the last resort of treatment options, resistance to it commonly leads to more severe complications and increased mortality (Capone, Giannella et al. 2013).

Deletions, insertions or mutations in several chromosomal genes affecting the composition of the cell wall, thus preventing the efficient binding of the drug, can lead to different levels of decreased colistin susceptibility (Olaitan, Morand et al. 2014). A transferable plasmid-derived colistin resistance gene mcr-1 discovered in China and subsequently found worldwide could be mediating this emergence (Liu, Wang et al. 2016; Skov and Monnet 2016).

It is believed that specific details on antibiotic resistant profiles of *E. coli* isolated from chicken in the country is useful for better application of antibiotics use for active control of disease caused by pathogenic *E. coli* in chicken. Therefore, the purpose of this study is to determine the resistance profiles especially colistin of *E. coli* strains isolated from chicken fecal samples in Gaza strip.

Materials and Methods

Sampling and sample duration

One hundred and ten fecal samples were collected from broiler chicken raised in various farms in Gaza strip. Samples were collected in sterile cups. Cups were properly labeled and placed in an ice box and transported to the lab within two hours of collection. The study was conducted during the period from January to March 2018.

Isolation of *E. coli* bacteria

A loop was inserted into the sample and used to streak MacConkey agar (Mac) and Eosin Methylene Blue agar (EMB) plates. The plates were incubated at 37°C for 24h. After incubation, all lactose fermenting (pink/green metallic sheen) colonies were picked and re-streaked onto MacConkey agar to obtain pure cultures. The plates were incubated again at 37°C for 24h. The isolates were then biochemically characterized and streaked onto the surface of Triple sugar iron agar (TSIA) for short-term storage and inoculated into Brain heart infusion broth (BHIB)/Glycerol for long term storage.

Biochemical characterization of *E. coli* isolates

Bacterial isolates were identified using standard biochemical tests (TSIA), sulfide-indole-motility, catalase, oxidase, and urease production, methyl red (MR) and Voges-Proskauer (VP) test, and citrate utilization as carbon source). An isolate was identified as *E. coli* if its TSIA reaction was Acid/Acid with gas, citrate, urease, H_2S and VP negative, while positive for motility, indole and MR.

Assay for antimicrobial drug resistance of the *E. coli* isolates

The bacterial isolates were subjected to *in vitro* antibiotic susceptibility test by disc diffusion method using Mueller-Hinton (MH) agar plates. Isolates were tested against 11 antimicrobials (Cetriaxone, Chloramphenicol, Ampicillin, Co-trimazol, Meropenem, Amikacin, Norfloxacin, Nalidixic acid, Neomycin, Cephalothin, and Colistin).

Antibiotic discs were placed on a pre-inoculated MH agar surface. The plates were incubated at 37°C overnight and diameter of the zones of growth inhibition was measured in mm (Singh, Praveen et al. 2009). The measurements were compared with zone size interpretative chart provided by the manufacturer and the isolates were graded as sensitive, intermediate and resistant as per CLSI (2018).

Data analysis

Data collected were summarized, tabulated and analyzed using Statistical Package for Social Sciences (SPSS) software. The results are presented through histograms, tables and pie charts. Multiple Antibiotic Resistance index (MAR index) was calculated for each isolate.

Results

Bacterial isolates and identification results in Selective media

A total of 110 samples were collected from Gaza strip farmers consisted of two source including Middle governorate (n=30) and Gaza governorate (n=80). On EMB culture growth characteristics of presumptive *E. coli* is depicted in figurer (1) with colonies showing a characteristic green metallic sheen. On MacConkey Agar media, *E. coli* culture was grown as shown in figure (1). Lactose fermenter; flat, dry, pink colonies with a surrounding darker pink area of precipitated bile salts. All 110 samples (100%), produced characteristic *E. coli* isolates on EMB agar and MacConkey Agar media.

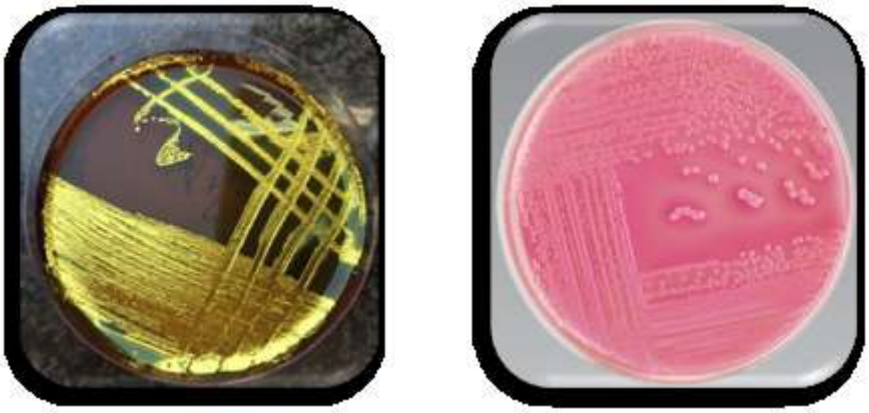

Figure (1): Growth characteristic (green sheen) of isolated *E. coli* in EMB media, characteristic (pink color) of isolated *E. coli* in MacConkey Agar media.

Antibiotic susceptibility profiles of *E. coli* isolates

The sizes of zones of inhibition of every antibiotic disc was measured in millimeter and compared with the interpretive standards of CLSI 2018 for *Enterobacteriacae*. Isolates were classified as sensitive, intermediate or resistant to the antibiotic. Different isolates produce various susceptibility patterns (Table 1).

Table (1): Resistance profiles of *E. coli* isolates

Antimicrobial	**Frequency**					
	R		S		I	
	N	%	N	%	N	%
Amikacin	1	0.9	109	99.1	0	0
Ampicillin	110	100	0	0	0	0
Cephalothin	95	86.4	5	4.5	10	9.1
Ceftriaxone	34	30.9	74	67.3	2	1.8
Chloramphenicol	48	42.6	59	53.6	3	2.7
Colistin	16	14.5	94	85.5	0	0
Co-trimazol	74	67.3	35	31.8	1	.9
Meropenem	5	4.5	97	88.2	8	7.3
Nalidixic acid	58	52.7	45	40.9	7	6.4
Neomycin	19	17.3	88	80	3	2.7
Norfloxacin	17	15.5	80	72.7	13	11.8

Resistance to Ampicillin was detected in all 110 (100%) isolates, followed by Cephalothin (86.4%), Cotrimazol (67.3%), Nalidixic acid (52.7%), Chloramphenicol (43.6%), Ceftriaxone in 34 (30.9%), Neomycin in 19 (17.3%), Norfloxacin in 17 (15.5), Colistin in 16 (14.5%), Meropenem in 5 (4.5%), Amikacin in 1 (.9%), of each isolates.

The results clearly present an alarming and disturbing numbers about the resistance pattern of *E. coli* isolated from poultry farms in Gaza strip. Table (2), illustrate the distribution of multi-drug resistance among *E. coli* isolates. Of the tested isolates, 76.4% (84) were multidrug resistant (MDR), and showed resistance to three or more antibiotics from the 11 applied antibiotics. One isolate was resistant to all tested antibiotics. The resistance profile of MDR isolates ranged from 3 to 9 antibiotics. Most isolates showed resistance to five or more antibiotics.

Table (2): Multi Drug Resistant *E. coli* isolates

	Frequency		**MARI value**
Valid	N	%	
Multidrug resistant strains	84	76.4	> 0.3 (0.3-0.8)
Non multidrug resistant strains	26	23.6	< 0.3
Total	110	110	

Discussion

One reason for choosing *E. coli* is that in recent years, mcr-1-harboring *Enterobacteriaceae* isolates were identified in food-producing animals, foods, aquatic environments, and humans (Liu, Wang et al. 2016). In this study, we report for the first time, to the best of our knowledge, the isolation of colistin-resistant *E. coli* strains in chicken fecal materials in Gaza.

There are no published studies describing antimicrobial resistance in poultry and chicken in Gaza strip. Our work represents a baseline

contribution toward understanding the interplay between antimicrobial resistance and colistin resistance on farms in Gaza strip.

Alarms were triggered in the microbiology as well as health communities in late 2015 when the first plasmid mediated mobile gene mcr-1 for colistin-resistance was identified in China. Soon after, the mcr-1 gene has been reported in *Enterobacteriaceae* from humans and food producing animals in various countries and continents (Lei, Wang et al. 2017). Colistin represent the last line against fatal infections by multidrug-resistant Gram-negative pathogens (Gao, Hu et al. 2016). Resistance to colistin or its evolution in commensal or potentially pathogenic bacteria of public health significance is an important health threat.

In this study, of the 110 isolates tested, 16 *E. coli* isolates, were colistin resistant using disk diffusion method. Emerging resistant strains of *E. coli* are potentially linked to un-justified use and misuse of antimicrobials in animal farms. In this study, a notable observation was that ampicillin and Cephalothin demonstrated very low *in vitro* antibacterial activity (0.0%, 14% respectively) against *E. coli* isolates. These results are lower than those reported in a study in Southern Vietnam (Nguyen, Nguyen et al. 2016).

This result was compatible with the result obtained by Nguyen, Nguyen et al. (2016), where they showed that the least effective drugs included ampicillin (97.8%) for same bacteria (Nguyen, Nguyen et al. 2016). Resistance to Amikacin and chloramphenicol was detected in 1 (0.9%), and 48 (42.5%) isolates respectively, This result is compatible with result of a study conducted in China which reported 6.8%% of the *E. coli* isolated from food animals samples to be resistant to Amikacin (Saputra, Jordan et al. 2017). This low rates of resistance to amikacin may be because amikacin is not approved for use in food animals (Dai, Lu et al. 2008). Although chloramphenicol has been banned for use in food animals in most countries, a similar high level of resistance was reported in chickens from China in 2010 (51.8%) and more than double the level found in 1993 (23.2%) (Chen, Zhang et al. 2014).

In our study there was no clear explanation for these high levels of resistance but it might be related to the use of chloramphenicol, for treat animal's respiratory infections and thus could have been introduced into many livestock operations. Florfenicol a derivative of chloramphenicol is reportedly used in veterinary medicine in Gaza,

therefore, cross-resistance may explain this relatively high resistance to chloramphenicol (White, Hudson et al. 2000).

The result obtained by same study, reported 22.2% of the isolated strains to be resistant to colistin. This result is incompatible with our result for colistin resistance (14.5%) (Nguyen, Nguyen et al. 2016). Another study conducted in Brazil reported 19.5%% of the *E. coli* isolated from chicken meat samples to be resistant to colistin (DF Monte, Fernandes et al. 2017).

The result obtained by study in France, reported 79 colistin-resistant *E. coli* isolates recovered from diseased pigs between 2009 and 2013 (Delannoy, Le Devendec et al. 2017). Four colistin resistant *E. coli* isolates were recovered from a dog (2 strains), a turkey (1 strain) and a chicken (1 strain) (Villa, Salinas et al. 2018).

Our study has limited the finding to phenotyping of colistin resistance and did not screen for the gene (mcr-1, mcr-2 and mrc-3), which are involved in colistin resistance. The result obtained by Trung et al., (2017), reported (59.4%) *mcr-1* in fecal samples from chickens (Trung, Matamoros et al. 2017). Transmission of colistin-resistant *E. coli* from a domesticated pig (Du, Chen et al. 2016) and companion animals (Zhang, Doi et al. 2016) to humans has been documented.

However, no studies on fecal presence of plasmid-mediated colistin resistance genes (mcr-1 to -5) or *E. coli* colistin resistance in camels were found (Rhouma, Bessalah et al. 2018). In another study two *E. coli* and one *Salmonella* Typhimurium variant Copenhagen were shown to be MDR, including resistance to colistin, with one *E. coli* and the *Salmonella* carrying the mcr-1 gene (Anjum, Duggett et al. 2016).

In our study, 76.4% (84) of the tested isolates were multidrug resistant (MDR). This result was compatible with the result conducted in America reported most *E. coli* isolates exhibited an MDR phenotype. Similarly, a higher prevalence of resistance was found among poultry fecal samples (Van den Bogaard, London et al. 2001). This is also consistent with the findings in a previous report (Lu, Dai et al. 2010) and a national surveillance study which showed high levels of MDR *E. coli* isolates in chickens (89.20%; 6,751/7,568) and pigs (90.00%; 6,806/7,562) in China (Zhang, Shen et al. 2017).

MDR bacterial strains of animal origin may spread into the human population by direct contacts and through food from animal sources. The high levels of antimicrobial resistance amongst the *E. coli* isolates

in our study is generally consistent with those reported in studies conducted in other areas of Gaza strip.

Given the potentially serious consequences of the spread of the colistin resistance from food production animals to humans, prudent use of antimicrobial drugs in animal production should be promoted and enforced locally, even in small-scale and household farms.

Conclusions and Recommendations.

The data presented in this study demonstrated the presence *E. coli* carrying colistin resistance in 14.5% of the isolates. Monitoring of veterinary drugs use and abuse in Gaza strip seem to be a priority. It is recommended to conduct further studies aiming at finding the patterns of antimicrobial resistance obtained from domestic and wild animal fecal samples. Genetic detection of the mcr-1 and mcr-2 genes which are the gene encoding a phosphoethanolamine transferase, which confers resistance to Colistin would provide more useful findings on Colistin resistance.

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