

High prevalence of antimicrobial resistance among Gram-negative bacteria isolated from poultry

Abdelraouf A. Elmanama¹, Mariam R. Al-Reefi¹,
Mohammed A. Albayoumi², Alaa M. Marouf¹,
Islam F. Hassona³

- 1 Medical Laboratory Sciences Department, Faculty of Health Sciences, Islamic University of Gaza, P.O Box 108, Gaza, Gaza strip, Palestine.
- 2 Veterinary Services, Ministry of Agriculture, Gaza, Palestine.
- 3 Al-Shifa Hospital, Ministry of Health, Gaza, Gaza strip, Palestine.

Abstract

Background: Infection with Multidrug resistant bacteria (MDR), such as *Escherichia coli* and *Salmonella* spp. are considered as threat to the human health care system. In recent years, these MDR bacteria have been found increasingly inside and outside the hospital environment. Food animals (meat and poultry) are increasingly colonized with MDR bacteria, thus posing an additional concern. This study is intended to determine susceptibility and resistance pattern of pathogenic Gram-negative bacteria isolated from rectal swabs of chicken against 16 antibiotics.

Methods: A total of 216 cloacal swab samples from Gaza strip poultry farms and 87 (frozen and fresh meat samples obtained from slaughter houses and retails) were collected over the period from June 2017 to June 2018. Isolation and identification of organisms were achieved using standard bacteriological techniques. Antimicrobial susceptibility test was performed according to standard protocols.

Results: A total 360 *Enterobacteriaceae* isolates, and 56 Gram-negative non fermenter were recovered. The predominant *Enterobacteriaceae* isolate was *Citrobacter* spp. (22.6%), followed by *Enterobacter* spp. (17.6%) and *E. coli* (16.5%). High rates of resistance against ampicillin (85.4%) and trimethoprim/sulfamethoxazole (80.1%) followed by chloramphenicol (74%) were recorded. Few samples were positive for *Salmonella* spp. and *Shigella* spp. Of the tested *Enterobacteriaceae* isolates, 94.7% were multidrug resistant (MDR), and 31.4% of non-fermenting bacilli (NFB) were MDR. Carbapenem resistance was found to be high among isolates; 51.9% for imipenem and 1.8% for meropenem.

Contact information:

Abdelraouf A. Elmanama.

 elmanama_144@yahoo.com

Conclusion: This study suggests that presence of high rates of MDR bacteria isolates in chickens constitutes an important reservoir of antimicrobial resistance in Gaza-Palestine which is a major public health concern.

Keywords

Multi-Drug Resistance; Antimicrobial Resistance; Poultry; Gaza- Palestine.

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Background

All forms of life clearly play an important role within the context of the One World, One Health according to the concept of World Health Organization. Food animals can serve as sources for infectious diseases for humans resulting from direct contact or environmental contamination. In addition, food animals can also transmit pathogens to other animal species directly and indirectly through environmental dispersal [1].

Escherichia coli is one of the most common intestinal commensal organisms found in human and many animals and therefore it is widely disseminated in the environment. Investigating potential pathogenic *E. coli* strains and/or antibiotic resistance may provide information regarding human activity in select ecological niches [2]. Bacteria may acquire resistance to antibiotics under selective pressure, but they may also become resistant to antibiotic without direct exposure to an antibiotic through horizontally mobile elements including conjugative plasmids, integrons and transposons [3]. These genetic elements can frequently transfer antibiotic resistance genes from one bacterium to another through the intestinal tract of human and animals [4].

Monitoring the prevalence of resistance among indicator bacteria such as *E. coli* and enterococci in different populations such as animals, patients and

healthy peoples, makes it feasible to compare the prevalence of resistance and to detect the transfer of resistant bacteria or resistance genes from animals to humans and vice versa [5]. However, few reports about the prevalence rates of antimicrobial resistance in *E. coli* and enterococci in food animals have been published [6].

In several countries, antibiotics such as penicillin, erythromycin and tetracycline are approved for growth promotion as well as therapeutic use in animals. Many of the antibiotics that are given to animals are closely related to medically important human drugs. Thus it is possible that the indiscriminate use of antibiotics of non-human use can lead to the development of resistance which could then be passed to human pathogens [7].

In Gaza strip, clinical studies have shown high and alarming rates of resistance [8-10]. Only one study has reported occurrence of colistin resistance (16%) among *E. coli* from poultry which will be published soon. Data concerning resistance among isolates from food animals are scarce.

This study was performed with the aim of investigation the occurrence of antimicrobial resistance among Gram-negative bacteria recovered from poultry in Gaza strip.

Material and Methods

Samples collection and transportation

The present research was conducted during the period from June 2017 to June 2018. A total of 216 cloacal swabs were collected from broiler chickens (two swabs from each chicken; one for *Salmonella* and *Shigella* isolation and the second for other Gram-negative isolation) and 87 meat samples (100grams each) were collected from broiler slaughterhouses and shops as fresh and frozen meat. Samples were obtained from different locations in Gaza (Rafah, Khan Younis and the Middle governorates). Samples were collected aseptically, kept in an icebox and transferred to the Microbiology Laboratory at the Islamic University of Gaza.

Microbiological investigation

Meat samples

A 25 g meat sample was homogenized at low speed in a sterile blender with 225 ml peptone water (0.1 %) for 2 minutes. This constituted the 10^{-1} dilution. MacConkey agar and Violet Red Bile Agar (VRBA) plates (for gram negative bacteria isolation) were streaked by a loopful of the 10^{-1} meat dilution. Well isolated bacterial colonies from both plates were picked and purified by streaking onto fresh media. The isolates were then identified by colony morphology and growth characteristics.

Cloacal swabs

One cloacal swab was inoculated onto pre-labeled Blood agar, MacConkey agar, and VRBA plates. The inoculated plates were incubated at 37 ± 0.5 °C for 24 hours. The other swab was used to inoculate a tube of Selenite-F broth for *Salmonella* and *Shigella* isolation.

Isolation of *Salmonella* and *Shigella* spp.

A 0.1 ml from the pre-incubated samples in peptone water and selenite F broth was streaked onto (Xylose Lysine Deoxycholate agar & *Salmonella Shigella*

agar) and incubated at 37° C for 24 hr. Following incubation, lactose non fermenting colonies with or without black centers were streaked onto urea spot test. Typical *Salmonella* and *Shigella* colonies on XLD and SSA that are negative urease test were subcultured into triple sugar iron agar slant and incubated for 24 hr at 37°C then identified using API 20E (bioMerieux, France).

Isolates identification

Confirmed gram negative isolate were identified biochemically using API 20E kit. Gram stain, growth on MacConkey, Triple sugar iron agar and oxidase tests were also used according to Krumperman [11].

Antibiotic Susceptibility Tests

The disc diffusion test was done for all isolates based on Clinical Laboratory Standard Institute guidelines [12]. Commercial Antimicrobial impregnated discs were dispensed on the surface of cultures of Muller-Hinton agar and incubated at 35°C for 20 hrs. The diameters of the zones of inhibition were recorded to the nearest mm and classified as resistant, intermediate, or susceptible according to established interpretive chart [12].

The Multiple Antibiotic Resistance (MAR) index

MAR (Multiple antibiotic resistance) index provides useful information for the evaluation of a health risks [13]. MAR index value higher than 0.2 is regarded as MDR. An isolate was regarded as MDR if they were resistant to one or more antibiotics of three different classes

Statistical Analysis

The data were analyzed with Statistical Package for the Social Sciences (SPSS), version 22.0 software (Microsoft SSPS, USA).

Results

A total of 360 *Enterobacteriaceae*, and 56 non-fermenting bacilli (NFB) were isolated from the three sampling locations (Table 1).

The results of the identification of isolates showed that the predominant isolate among *Enterobacteriaceae*, was *Citrobacter* spp. (22.6%), followed by *Enterobacter* spp (17.6%) and *E. coli* (16.5%) (Figure 1).

In general, amikacin and meropenem showed the highest activity among all the tested antimicrobial, while tetracycline, ampicillin and trimethoprim/sulfamethoxazole were the least active drugs as show in Table 2.

Table 3. Shows the result of the percentage resistance of the *Enterobacteriaceae* isolates to each of the tested antimicrobials. A total of 416 isolates were tested against sixteen commercially available antibiotics. Among the tested isolates, none of the *Enterobacteriaceae* and NFB were susceptible to all antibiotics. The majority of the *Enterobacteriaceae* and Gram-negative non-fermenter isolates showed variation in their susceptibilities to different antimicrobials. Among *Enterobacteriaceae*, resistance to amikacin is 0.3% and high rates of resistance were observed against tetracycline (94.1%). High rates of resistance against ampicillin (85.4%) and trimethoprim/sulfamethoxazole (80.1%), followed by chloramphenicol (74%) as shown in Table 2.

In general, almost all isolates showed high percentage of resistance to tetracycline, ampicillin, and trimethoprim/sulfamethoxazole. *Klebsiella* spp., *Citrobacter* spp., *Morganella* spp., *Salmonella* spp., and *Shigella* spp. were the only species that showed absolute susceptibility to meropenem. This pattern was so different with imipenem, wherein, almost all isolates exhibited resistance with the notable exception of *Shigella* spp. (Table 3).

Table 4 shows the lowest resistance rates among NFB isolates to azithromycin (0%), amikacin (2%) and meropenem (2%).

Most *Enterobacteriaceae* isolates (94.7%) had a MAR index higher than 0.2, while the MDR among NFB was lower (31.4%) (Table 5).

Table 1. Distribution of bacterial isolates from selected poultry farms at three locations in Gaza.

Gram Negative isolates	Total	Governorate					
		Gaza		South Gaza		The Middle	
		N	%	N	%	N	%
<i>Enterobacteriaceae</i>	360	158	43.9	45	12.5	157	43.6
Non-Fermenters (<i>Pseudomonas</i> sp.)	56	0	0	24	42.9	32	57.1
Total	416	158	38	69	16.6	189	45.4

Figure 1: Percentage of *Enterobacteriaceae* species isolated from poultry meat and cloacal swabs.

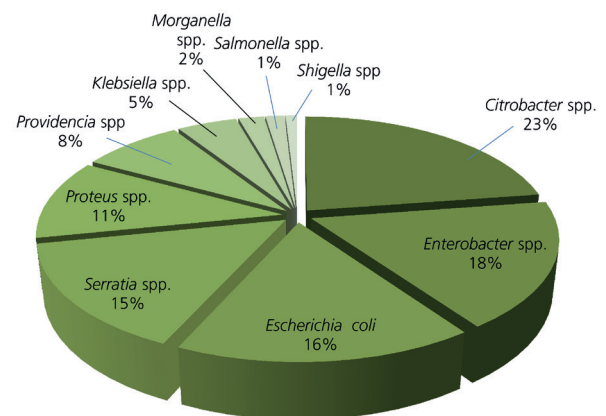


Table 2. Over all resistance rates of Gram-negative bacterial isolates to the tested antimicrobial agents.

Antimicrobial	No.	Resistance	
		N	%
Amikacin	338	1	0.3
Ampicillin	316	270	85.4
Ceftazidime	340	150	44.1
Ceftriaxone	292	90	30.8
Cefuroxime	324	74	22.8
Chloramphenicol	331	245	74
Ciprofloxacin	338	160	47.3
Gentamicin	341	160	46.9
Imipenem	335	174	51.9
Meropenem	339	6	1.8
Nitrofurantoin	280	132	47.1
Tetracycline	324	305	94.1
TM/SXT	322	258	80.1

TM/SXT: Trimethoprim/Sulfamethoxazole.

Table 3. Distribution percentage of antimicrobials resistance among *Enterobacteriaceae* species.

Antimicrobial	Resistance of <i>Enterobacteriaceae</i> isolates									
	No./%									
	<i>Providencia spp.</i>	<i>Enterobacter spp.</i>	<i>Serratia spp.</i>	<i>Klebsiella spp.</i>	<i>Citrobacter spp.</i>	<i>Proteus spp.</i>	<i>E. coli</i>	<i>Morganella spp.</i>	<i>Salmonella spp.</i>	<i>Shigella spp.</i>
Antimicrobial	27	60	51	16	77	38	56	7	3	3
Amikacin	0	0	0	0	0	0	1	0	0	0
Ampicillin	9	19.5	13.9	5.3	20.7	9.4	19.1	1.9	0.8	0.8
Ceftazidime	8.8	17.4	14.8	6	18.8	14.1	15.4	2.7	0.7	0
Ceftriaxone	11.3	19.3	17	5.7	21.6	9.1	10.2	4.5	0	1.1
Cefuroxime	13.9	19.4	5.6	7	27.8	13.9	8.3	2.8	0	1.4
Chloramphenicol	8.3	19.2	15	5.5	20.5	8.7	18.3	2.5	0.4	0.8
Ciprofloxacin	7	24.7	12	5.7	23.4	4.4	20.3	1.3	0	0.6
Gentamicin	10	21.3	8.1	8.1	22.6	6.3	20	2.5	0	1.3
Imipenem	7.1	11.7	18.7	6.5	21.7	16.4	13.5	2.3	1.2	0.6
Meropenem	33.3	16.7	16.7	0	0	16.7	16.7	0	0	0
Nitrofurantoin	5.3	11.4	21.4	7.6	17.6	20.6	11.5	3.1	0.8	0
Tetracycline	7.4	18.8	15.1	4.7	21.8	11	16.7	1.7	1	1
Trimethoprim/ Sulfamethoxazole	7.1	20.1	15.7	5.1	23.6	9.5	14.6	2.8	0.8	0

Table 4. Distribution of resistance rates among Gram negative non-fermenting bacilli bacterial isolates (N=51) to tested antimicrobial agents.

Antimicrobial	Isolates	Resistance
	N	%
Amikacin	1	2
Azithromycin	0	0
Cefepime	2	3.9
Ceftazidime	11	21.6
Gentamicin	15	32.6
Imipenem	6	11.8
Levofloxacin	13	25.5
Meropenem	1	2

Table 5. Multi drug resistance in Gram-negative isolates.

Valid	Isolates	
	Gram negative bacilli	Gram negative non-fermenting bacilli
	%	%
MDR	94.7	31.4
Non MDR	5.3	68.6
Total	100	100

Discussion

The World Health Organization (WHO) has recognized that antimicrobial resistance is a global problem that calls for a global response. Consequently, WHO issued the global principles for the containment of antimicrobial resistance in animals intended for food. The WHO global strategy for the containment of

antimicrobial resistance where some interventions were recommended that hopefully will enable local authorities to slow down the emergence and reduce the spread of resistance in diverse settings [14]. Transfer of resistant bacteria between animals and humans through food products has been documented and can pose a threat to public health [15].

This study has attempted to determine the resistance patterns of Gram-negative bacteria isolated from poultry in Gaza, Palestine. The overall percentage of *E. coli* among *Enterobacteriaceae* was 16%, this is low when compared to the prevalence rate of 37.86% obtained by Rahman *et al.*; [16] and 21.09% by Rahman *et al.*, [17]. Even very much higher results were reported with a prevalence rate of 76% by Barua *et al.*, [18], and (78.9%) by Jakkaria *et al.*, [19].

Antimicrobial resistance among *Enterobacteriaceae* and NFB isolates was determined against 16 commonly used antibiotics. Although the pathogenicity of these isolates was not examined, the genes responsible for multi-drug resistance may be transmitted to people by food and may result in serious public health hazards.

In our study, only one isolate of *E. coli* was resistant to amikacin, this is not comparable to the prevalence rate of 37.7% obtained by Rahman *et al.* [16] and differ greatly from the findings of Arya *et al.* (2008) who reported that 80% of Shiga toxin producing *E. coli* (STEC) strains were resistant to amikacin, tetracycline (63%), and ciprofloxacin (20%) [20].

The efficacy of tetracycline against *Enterobacteriaceae* isolates was shown to be the least effective as evident by the high resistance percentages (94.1%). This result is close to the results obtained in Indonesia by Yulistiani *et al.* (69.9%) [21] and very much higher than the percentage reported by Azevedo *et al.* (52.38%) [22]. High resistance to tetracycline in this study can be interpreted by the extensive, uncontrolled, widely and long use of tetracycline in poultry farms as a growth promoter

agent, and for disease prevention. Improper and intensive use of antibiotics in poultry may be one trigger of antibiotic resistance particularly to antibiotics that are often used on the chicken farms. Bacteria can become resistant to tetracycline by at least three mechanisms; enzymatic inactivation of tetracycline where an acetyl group is added to the molecule causing inactivation of the drug, efflux resistance gene encodes a membrane protein that actively pumps tetracycline out of the cell, and ribosomal protection which blocking tetracycline from binding to the ribosome [23, 24].

Our results showed that 94.7% and 31.4% of the tested *Enterobacteriaceae* and NFB isolates were MDR. The high prevalence of antimicrobial resistance obtained can be explained by the common practice of using of antimicrobials as prophylactics and growth enhancers in poultry production. Unfortunately, the authors were not able to find any published data regarding the amount of antimicrobials usage in Gaza, however, one study from Gaza strip reported malpractices and abuse of antimicrobials in poultry farms [25].

The result of the antibiotics resistance patterns for *Enterobacteriaceae* obtained by Yulistiani *et al.* [21], showed high level of multi-drug resistant bacteria, yet lower than obtained in this study. As many as 61.1% of *Enterobacteriaceae* isolates were MDR to at least three unrelated antimicrobial agents.

In our study, the dominant resistance was to tetracycline, and ampicillin in 94.1%, 85.4%, respectively. This result is higher than the results obtained by Azevedo *et al.* who reported resistance of 32.8% for Ampicillin and only 4% for tetracycline [22].

Carbapenem-resistant *Enterobacteriaceae* (CRE) isolates are a serious public health issue due to limited therapeutic options to treat such infections. CRE isolates have been predominantly isolated from humans and environmental samples and they are rarely reported among isolates from poultry [26].

Meropenem resistance among all isolates was low (1.8%) while higher resistance against imipe-

nem was demonstrated (51.9%). These results are similar to those obtained by Webb *et. al.*, who investigated the susceptibility of the bacterial isolates to the carbapenems including imipenem, and meropenem, and showed that out of the 360 *Enterobacteriaceae* isolates recovered, 51.9% and 1.8% were resistant to imipenem, and meropenem, respectively [27]. Based on the results described herein, CRE appear to be high among chicken.

Recent studies have highlighted the global emergence of CRE isolates in both livestock [28] and companion animals [29]. In particular, recent studies demonstrating the isolation of carbapenemase-producing *E. coli* (NDM-1 and OXA-48) and *Klebsiella pneumoniae* (OXA-48) from clinical infections in dogs [29] and questions about the veterinary use of carbapenems are raised [30]. Carbapenemase producing organisms were isolated from 5 of 127 (4%) products tested [31].

This study shows that both *Salmonella* and *Shigella* isolated from poultry samples were highly susceptibility to almost all tested agents and none of them was resistant to meropenem.

A study conducted in one dairy farm in France, 2010, samples taken from 50 dairy cows were tested. Nine of the samples were positive for carbapenemase producing (CP) in *Acinetobacter* spp. isolates [32]. While a survey on chicken and duck farms and pig slaughterhouse in Eastern China showed that nine isolates from different bacterial genera (*Stenotrophomonas*, *Myroides*, *Chryseobacterium*, and *Acinetobacter*) were non-susceptible to carbapenems [28]. From longitudinal studies conducted in swine and broiler farms in Germany [33], two fattening pig farms and broiler farm were found to be positive for carbapenemase producing *Salmonella* spp. One of these farms was also positive for CP *E. coli*.

Carbapenems are not licensed for use in food-producing animals in Europe and other parts of the world. Therefore, one simple and effective control approach to minimize the further emergence and

possible spread of carbapenem-resistant strains via the food chain would be to continue to ban the use of carbapenems in food-producing animals [34]. There are no reports of using carbapenems in poultry in Gaza, and one explanation for this high rate of carbapenem resistance among poultry isolates is that human strains may have colonized those poultry farms. This result can be supported by the fact that many poultry farms are found in residential areas and there are no infection control measures practiced by poultry farmers.

In conclusion, MDR among Gram-negative bacteria isolated from chicken cloacal swabs and chicken meat was found in high rates in Gaza. Carbapenem resistance was also prominent most especially for imipenem. This result requires further investigation to track the source of carbapenem-resistant bacteria. The Ministry of Agriculture in Gaza should implement monitoring programs and control measures to combat this serious public health issue.

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