Antimicrobial resistance among *Campylobacter* isolates from poultryand human of different localitiesin Egypt.

Khalil, M.R, Nashwa O. Khalifa*; Mona M. Sobhy**
and Nagwa S. Rabie***
Dept. of poultry, Animal Research Inst. El-Mansoura Branch
** Dept. of Zoonosis, Fac. of Vet. Med.Benha Univ.*
*** Dept. of Reproductive diseases, ARRI, ARC.Giza, Egypt.*
****Dept. of Poultry Diseases, National Research Center. Dokki, Giza,Egypt.*

Abstract:
The present study was carried out to screen and analyze the characteristics of antibiotic resistance in *Campylobacter* strains isolated frompoultry and human in the poultry farms of different localities in Egypt. A total of 340 samples were taken from poultry and human from poultry farms and examined bacteriologically for isolation of Campylobacter organisms. Fifty-six (16.47%) samples were identified as *Campylobacter*-positive; 50 (14.71%) from poultry samples and 6(1.76%) from human samples using conventional method. The isolates were42 (12.35%) isolates for *C. jejuni* including 38 (12.67%) from poultry samples and4(10%) from human samples. Isolates for *C. coli* were 14 (4.12%) including 12 (4%) from poultry samples and 2 (5%) from human samples detected. All *Campylobacter* isolates were evaluated for their antibiotic susceptibilities.

Results of Antibiogram revealed that *Campylobacter* isolates were resistant to one or more of the antibiotics tested. Resistance was most frequently observed against streptomycin (96.4%) amoxicillin (94.6%), doxycycline (87.5%), Ampicillin (83.9%), nalidixic acid (85.7%), erythromycin and ciprofloxacin (82.1%). *C. jejuni* strains were often resistant to cephalothin (35.7%) than *C. coli* strains (42.8%). *C. coli* were sensitive to erythromycin and Streptomycin (100%). *C. jejuni* was an increase sensitive to amoxicillin and streptomycin (95.2%). The trend of resistance to gentamicin (28.6%) and tetracycline (50%) was observed for *C. jejuni.*

The present study provides an assessment of the occurrence of multidrug resistance of *Campylobacter isolates* from chicken samples collected from the poultry farms in different localities in Egypt. The antimicrobial resistance rates among these pathogens are clearly important in risk assessment and management. Further research is also needed to better understand the relationship between antimicrobial used in poultry and humans and the bacterial resistance in humans.

Introduction:

Antimicrobial resistance (AMR) is a serious threat to public health, leading to mounting health care costs, treatment failure, and deaths ***(Högberg, et al, 2014)***.*Campylobacter* enteritis orcampylobacteriosis in humans hadbeen implicated with the consumption of chicken meat and chicken products ***(CDC, 2005; Skirrow, 1998 and Tauxe, 1992)***.*C. jejuni* is the most frequent cause of food-borne bacterial gastroenteritis in the world. Poultryare the largest source of infection, with approximately 80% of poultry carcasses contaminated in the world. *C. jejuni* colonizes the chicken gut, primarily the large blind ceca at the distal end of the gastrointestinal tract to levels in excess of 10^9 CFU/g. *Campylobacter* is rapidly transmitted horizontally through broiler (meat-producing) flocks as a consequence of fecal shedding of the bacterium in the chicken ***(Humphrey et al, 2014)***

Campylobacter is a commensal constituent in the microflora of a wide range of animals, and has been isolated from numerous hosts including domestic and wild mammals, birds and reptiles ***(Nachamkin & Blaser, 2000 and Allos, 2001)***. The majority of campylobacteriosis in chicken is caused by *C. jejuni* and *C. coli*. Most of *Campylobacter* enteric infections are self-limited and do not require antimicrobial drug treatment. However, severe or long-lasting *Campylobacter* infections occur and may justify antimicrobial drug therapy ***(Girard et al, 2006 and Stern et al, 2003)***.

Antibiotic-resistant bacteria may keep recovering at all. Children; the elderly and those with weakened immune systems are particularly vulnerable because their immune systems are not as vigorous as those of healthy adults ***(Nawal Hassanin , 2011)***.

Resistance of Campylobacter to antimicrobial agents has increased substantially during the past 2 decades and has become a matter of concern in Campylobacter infections. Combined studies in humans and poultry have implicated the use of fluoroquinolones in poultry in the emergence of drug resistance ***(Davidson, 2004)***.

This study quantifies the occurrence of antimicrobial resistance and investigates temporal trends among *C. jejuni* and *C. coli* isolates from poultry, by considering this in the context of a phylogeny for *C. jejuni*and *C. coli*. Also this study wasdesigned to investigate the extent to which increases in antimicrobial resistance to improve diagnostic accuracy and treatment of *Campylobacter* microorganisms in poultry.

Materials and methods

1- Samples:

A total of 340 samples from human and poultry including "40 human stool samples, 140 fecal droppings and 160 intestinal contents" were collected from different localities in Egypt (Table, 1).

2- Bacteriological Examination:

The collected samples were examined bacteriologically for isolation andidentification of *Campylobacter* as follows:The collected samples were inoculated into thioglucolate broth incubated at 37°C for 24 hrs. and examined under phase contrast microscope for isolation of *Campylobacter*species. The positive samples were inoculated oncharcoal cefoperazone desoxycholate agar (CCDA) medium. The plates were incubated at 37°C for 72 hoursunder special microaerophilic condition (85 % nitrogen,5% oxygen and 10 % carbon dioxide). The suspectedcolonies were identified according to ***Murray et al (2003).***

3- *Antibiotic Sensitivity Testing*:

Campylobacter isolates from all samples were evaluated for the susceptibility to 10 antimicrobial drugs :Ampicillin (10 µg),Cephalothin (30 µg), nalidixic acid(30 µg), ciprofloxacin(5 µg), erythromycin(15 µg), amoxicillin(10 µg), gentamicin(10 µg), tetracycline (30 µg), doxycycline (10 µg) and streptomycin(10 µg)) by the agar diffusion method on Mueller-Hinton agar enriched with 5% sheep blood by using antibiotic disks. Multidrug resistance was defined as resistance to >2 antimicrobial drugs.The agar disk diffusion technique was adopted according to ***Quinn et al (2002).*** Theresults were interpreted according to the ***National Committee for Clinical Laboratory Standards (2002).***

4- Statistical Analysis

Differences between proportions and isolation rates were tested by χ^2 and Fisher exact tests. Means were compared with Student and Fisher tests. Patterns of antimicrobial resistance were analyzed.

Table (1): *Samples collected from poultry farms and human.*

Localities	Type of Samples			No. of samples
	Poultry samples		Human samples	
	Faecal droppings	Intestinal contents	stool	
1-Cairo	30	30	10	70
2-Giza	32	35	8	75
3-Kaliobia	33	37	9	79
4-Monefia	25	30	7	62
5-Fayoum	20	28	6	54
Total	**140**	**160**	**40**	**340**

Results

Fifty six (16.47%) samples out of 340 samples were detected as *Campylobacter*-positive which classified as 48 (14.12%) from poultry samples and 8(2.35%) from human samples using conventionalplating method including 42 (12.35%) isolates for *C. jejuni* and 14 (4.12%)isolates for *C. coli* detected (tables, 2& 3).

Campylobacter isolates were inconstantly resistance to streptomycin, and a high number of isolates which resistant to gentamicin was recorded in poultry. Isolates were less frequently resistant to tetracycline but more often resistant to amoxicillin. For gentamicinresistance among *C. jejuni* strains was (28.6%)but much more frequent for *C. coli* (35.7%) of poultry isolates(table, 4).

Table (4) revealed that*C. jejuni* strains were often resistant to cephalothin(35.7%)*C. coli* strains were (42.8%)). *C. coli*were highlyresistant to erythromycin andstreptomycin (100%). *C. jejuni*wasanhighly resistant to amoxicillin (97.6%). Similar trends were observed for *C. jejuni*resistant tostreptomycin (95.2%). The trend of resistance to gentamicin (28.9%) and tetracycline (50%)was observed for *C. jejuni.*

Table (2): *Incidence of C. jejuni and C. coli in different collected samples.*

Type of Samples	No. of samples	Campylobacter isolates*		Total of +ve Campylobacter isolates*
		C. jejuni	C. coli	
- Faecal droppings	140	18(12.86%)	5(3.57%)	23(16.43%)
- Intestinal contents	160	20(12.5%)	7(4.38%)	27(16.88%)
- Human stool	40	4(10%)	2(5%)	6(15%)
Total	**340**	**42 (12.35%)**	**14 (4.12%)**	**56(16.47%)**

*=Total No. of isolates to No. of samples.

Table(3): *Results of Campylobacter species in examined samples at different localities.*

Localities	No. of samples	C. jejuni	C. coli	Total Campylobacter isolates*
1-Cairo	70	9(12.9%)	3(4.3%)	12(17.14%)
2-Giza	75	11(14.7%)	3(4%)	14(18.67%)
3-Kaliobia	79	12(15.2%)	4(5.1%)	16(20.25%)
4-Monefia	62	7(11.3%)	2(3.2%)	9(14.52%)
5-Fayoum	54	3(5.5%)	2(3.7%)	5(9.26%)
Total	**340**	**42 (12.35%)**	**14 (4.12%)**	**56 (16.47%)**

*=Total No. of isolates% to No. of samples.

Table (4): *Number and percentages of antimicrobial-resistant C. jejuni and C. coli strains isolated frompoultry andhuman samples.*

Antibiotic/μg	***C. jejuni* (n = 42)***	***C. coli* (n = 14)***	**Total (n = 56)***
Ampicillin10 μg	40(95.2%)	7 (50%)	47(83.9%)
Cephalothin30 μg	15 (35.7%)	6(42.8%)	21(37.5%)
Nalidixic acid30	35 (83.3%)	13 (92.9%)	48(85.7%)
μg**Ciprofloxacin** 5	34(80.9%)	12 (85.7%)	46(82.1%)
μg**Erythromycin**15	32 (76.2%)	14 (100%)	46(82.1%)
μg**Amoxicillin** 10	41 (97.6%)	12 (85.7%)	53(94.6%)
μg**Gentamicin** 10	12 (28.6%)	5 (35.7%)	17(30.4%)
μg**Tetracycline**30 μg	21 (50%)	11 (78.6%)	32(57.1%)
Doxycycline10 μg	38 (90.5%)	11 (78.6%)	49(87.5%)
Streptomycin10 μg	40 (95.2%)	14 (100%)	54(96.4%)

*: No. of isolates.

Table (5): *Number and percentages of antimicrobial-resistant C. jejuni and C. coli strains isolated from human samples.*

Antibiotic/μg	***C. jejuni* (n = 4)***	***C. coli* (n = 2)***	**Total (n = 6)***
Ampicillin10 μg	**4 (100%)**	**2 (100%)**	**6(100%)**
Cephalothin30 μg	**1 (25%)**	**1(50%)**	**2(33.3%)**
Nalidixic acid30	**3 (75%)**	**1 (50%)**	**4(66.7%)**
μg**Ciprofloxacin** 5	**3 (75%)**	**2 (100%)**	**5(83.3%)**
μg**Erythromycin** 15	**2 (50%)**	**2 (100%)**	**4(66.7%)**
μg**Amoxicillin** 10	**4 (100%)**	**2 (100%)**	**6(100%)**
μg**Gentamicin** 10	**1 (25%)**	**1 (50%)**	**2(33.3%)**
μg**Tetracycline**30 μg	**2 (50%)**	**1 (50%)**	**3(50%)**
Doxycycline10 μg	**3 (75%)**	**1 (50%)**	**4 (66.7%)**
Streptomycin10 μg	**4(100%)**	**2 (100%)**	**6(100%)**

*: No. of isolates

Discussion:

The antimicrobial resistance in *Campylobacter* isolated from poultry is widespread and may be increasing. Since poultry is considered to be one of the most important reservoirs of human *Campylobacter* infections, this pervasive resistancethe trend towards increasing levels of antimicrobial resistanceamong *Campylobacter* isolates from poultry has implications for containing outbreaks of drug resistant strains in humans ***(Norström et al, 2007)***.

From results presented in (tables2 & 3) it is clear that higher incidence of C. *jejuni* isolation was reported from faecal droppings of poultry (12.86%)followed by intestinal contents(12.5%) and human stool (10%), but *C. coli* isolates was higher inhuman stool (5%) followed by intestinal contents of poultry(4.38%) then faecal droppings(3.57%). Nearly similar results were reported in chicken by ***(Nawal Hassanain, 2011).*** On the other hand ***(Besterand Essack, 2008)*** reported higher incidence 49.6%of isolated *Campylobacter* from intestinal content samples of chickens.

Table 3 summarized theprevalence of *C. jejuni* and *C. coli* in different localities in Egypt; the prevalence of *C. jejuni* and *C.coli* in human and chicken samples in Cairo were 12.9% and 4.3% respectively but in Giza were14.7% and 4%, respectively. Theoccurrence of *C. jejuni* and *C. coli* in Kaliobia showedsignificantly higher than in Giza 15.2% for *C. jejuni*and 5.1% for*C. coli*. Lower incidences were recorded in Fayoum 5.5% for *C. jejuni* and 3.7% for *C. coli*.

Increased prevalence of*Campylobacter* in poultry has been associated with farm workers may carry *Campylobacter* from one flock toanother if they move between different flocks without changing clothes and boots. Many studies have indicatedthat the application of hygiene barriers significantlyreduced the prevalence of *Campylobacter* in chicken flocks ***(Cardinale et al, 2004)***.

*Campylobacter sp.*resistance to gentamicin was greater for *C. coli* (35.7%) than for *C. jejuni*(28.6%).Cephalothin (42.8%)and ampicillin (50%)were resistance for *C. coli* (Table, 4). Resistance to cephalothin(35.7%) and tetracycline (50%) for *C. jejuni* had the same pattern in *Campylobacter* isolates showed by (***Nagwa Rabie, 1992) and (Jorgen et al, 2001).***

Present investigation revealed resistance of the C. *jejuni*strains to gentamicin and cephalothinwere 28.6%and35.7% respectively. ***(Oza et al, 2003)*** recorded lower resistance rates. In contrary, ***(Abdalameer et al, 1999)*** reported that most of *C. jejuni* isolates were sensitive to gentamicin and cephalothin.In the present study, **6**isolates from human stool were prepared forsusceptibility testing to antimicrobial agents (Table,5). The highestpercentage of resistance was observedtoward ampicillin, amoxicillin and streptomycin (100%) andciprofloxacin (83.3%). Resistance towardserythromycin,doxycycline, and nalidixic acid,were (66.7%). The lowest frequency ofantibiotic resistance was observed towardgentamicin and cephalothin (33.3%).

Resistance among Campylobacter isolated from chicken is a potential hazard in that the resistance may occur in zoonotic pathogens such as *Campylobacter* species and so potentially reduce the effectiveness of antimicrobial treatment of food borne disease if contracted by humans ***(Franklin et al, 2000 and Hall et al, 2005)***.The present study showed there ishigh incidence of *Campylobacter* in chickensamples examined. This indicates thatchickens might be commonly contaminatedwith campylobacters; most of which wereantimicrobial-resistant. Thus, it might aserious health risk to consumers whoconsumed undercooked or post-cookingcontaminated chickens as antibiotics,namelyerythromycin or tetracycline, arenormally being prescribed in Campylobacteriosis in human cases asbloody diarrhea inpatients the treatmentin such cases will be compromised ***(Tang et al, 2009)***.

Finally, it can be concluded that since *Campylobacter* are zoonotic pathogens, resistance among isolates in animal and chicken reservoirs could have consequences for the treatment of infections in animals and humans. So, the antimicrobial resistance rates among these pathogens are clearly important in risk assessment and management.Further research is also needed to better understand the relationship between antimicrobial use in poultry and humans and thebacterial resistance in humans.

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مقاومة المضادات الحيوية لميكروبات الكامبيلوباكتر المعزولة من الدواجن و الانسان بمناطق مختلفة فى مصر

مصطفى ربيع خليل و نشوه عثمان خليفة* و منى محمد صبحى و نجوى سعد ربيع*****

قسم الدواجن - معهد بحوث صحة الحيوان – فرع المنصورة– مركز البحوث الزراعية

** قسم الامراض المشتركة – كلية الطب البيطرى بمشتهر – جامعة بنها*

*** قسم بحوث الامراض التناسلية – معهد بحوث التناسليات – مركز البحوث الزراعية*

**** قسم أمراض الدواجن – الشعبة البيطرية – المركز القومى للبحوث*

أجريت هذه الدراسة لفحص وتحليل خصائص عترات الكامبيلوباكتر المقاومة للمضادات الحيوية في سلالات معزولة من براز الانسان ودجاج في مزارع الدواجن بمناطق مختلفة في مصر.أخذت عدد٣٤٠عينة مختلفة شملت عدد ١٤٠ عينة من براز الدواجن و ١٦٠ عينة من الاحشاء الداخلية للدواجن وعدد ٤٠ عينة من براز الانسان وبالفحص البكتريولوجي التقليدى للمستنبتات البكتريولوجية للعينات،كانت نتيجة الفحوصات عزل عدد ستة وخمسون معزول للكامبيلوباكتر بنسبة(١٦,٤٧٪) منها ٦ (٧٦ر١%) من براز الانسان موزعة كالاتى:عدد ٤٢ معزولة للكامبيلوباكتر المعوى بنسبة (٣٥و١٢%) و عدد ١٤ معزولة للكامبيلوباكتر القولونى بنسبة (١٢و٤%). و تم زرع العينات الايجابية للكامبيلوباكتر وتقييم حساسيتها للمضادات الحيوية.
كشفت نتائج الحساسية للمضادات الحيوية لعترات الكامبيلوباكتر المعزولة أنها مقاومة لواحد أو أكثر من المضادات الحيوية التي تم اختبارها. وقد لوحظ في معظم الأحيان مقاومتها ضد الستربتومايسين (٩٦,٤٪) أموكسيسيلين (٩٤,٦٪)، الدوكسيسيكلين (٨٧,٥٪)، وحمض الناليديكسيك (٨٥,٧٪)، الأمبيسلين (٨٣,٩٪)، الإريثروميسين وسيبروفلوكساسين (٨٢,١٪) على التوالى.
كانت حساسية الكامبيلوباكتر المعوى غالبا مقاومة للسيفالوثين (٣٥,٧٪) أما عترات الكامبيلوباكتر القولونى كانت نسبة حساستها لنفس المضاد الحيوى(٤٢,٨٪).وقد أثبتت النتائج حساسية الكامبيلوباكتر القولوني للإريثروميسين والستربتوميسين بنسبة (١٠٠٪). وزيادة نسبة حساسية عترات الكامبيلوباكتر المعوى للأموكسيسيلين والستربتومايسين بنسبة (٩٥,٢٪). وقد لوحظ مقاومتها لمضادات جنتاميسين بنسبة (٢٨,٦٪) والتتراسيكلين بنسبة (٥٠٪).
وتقدم هذه الدراسة تقييما لحدوث مقاومة للأدوية المتعددة من العترات المعزولة من عينات الانسان والدواجن بمصر التي تم جمعها من مزارع الدواجن في مناطق مختلفة في مصر.ومعدلات مقاومة المضادات الحيوية للميكروبات بين هذه الجراثيم هامة جدا بشكل واضح في تقييم المخاطر وإدارتها وهناك حاجة إلى مزيد من البحث أيضا إلى فهم أفضل علاقة بين استخدام مضادات الميكروبات في الدواجن والبشروالمقاومة البكتيرية في الانسان.