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Antimicrobial Resistant *Campylobacter jejuni* Isolated from Humans and Animals in Egypt

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Abstract: A total of 310 animal samples including 50 fecal (droppings of broiler chickens) and 260 intestinal content (105 broiler chicken, 50 cattle, 55 buffalo and 50 sheep) and 48 human fecal samples was collected from the investigated food animal contacts from El-Monieb and El-Warak abattoirs and different animal and poultry farms at Giza Governorate, Egypt. Bacteriological examination of samples revealed that 17 samples (10.96%) including 5 (10%) and 12 (11.42%) from poultry fecal droppings and intestinal content samples, respectively and 8 (16.66%) out of the 48 human fecal samples were positive for *Campylobacter*. All the human and poultry *Campylobacter* isolates were biochemically identified to be *C. jejuni*. Results of the antibiotic susceptibility of the isolated *Campylobacter* strains showed that poultry *Campylobacter* strains displayed resistance of 64.71% to ampicillin, streptomycin and chloramphenicol and 58.82% to erythromycin and tetracycline. While, human strains resistance pattern was 87.5% to ampicillin, 75% to streptomycin and tetracycline, 62.5% to erythromycin and 50 % to chloramphenicol. It is recommended that control measures should be applied for decreasing antibiotic resistance in *Campylobacter* species.

Key words: *Campylobacter* · Antibiotic resistance · Food producing animals · Human contacts

INTRODUCTION

Campylobacteriosis is a worldwide zoonosis with an estimated 400 million cases per year worldwide [1]. The organisms are commonly found as commensal in the gastrointestinal tracts of all types of fowl, wild and domesticated cattle, domestic dogs and cats, rodents and many other animals. *Campylobacter* acquisition by animals occurs early in life and may lead to disease and death, but most animals become lifelong carriers [2, 3].

In general, people can become infected with *Campylobacter* spp. from contaminated meat by three different ways: (i) by handling raw meat and meat products, which is commonly reported in persons preparing the food or in inexperienced workers in processing plants; (ii) by consumption of raw or undercooked beef, hamburgers and sausages, salted, smoked, or slightly cooked pork; as well as raw fish and shellfish; e.g. clams; (iii) by consumption of foods that are usually eaten raw or without further cooking such as salads and breads, which may become cross contaminated [4].

C. jejuni and *C. coli* are recognized as the most common causative agents of bacterial gastroenteritis in the world and infections with these organisms occur more frequently than do infections due to *Salmonella* species, *Shigella* species, or *Escherichia coli* 0157:H7 [5]. *Campylobacter* species most commonly associated with diarrheal diseases in humans and are clinically indistinguishable [2]. Infections are generally self-limiting, with symptoms resolving in about 3 to 5 days [6]. Nevertheless, antibiotic therapy is required in immunocompromised patients, in case of bacteremia and in severe and long-lasting *Campylobacter* infections.

The spread of new generation of infections resistant to antibiotic treatments has serious consequences for public health. Antibiotic-resistant bacteria may keep people sick for long time and sometimes people are unable to recover at all. Children, the elderly and those with weakened immune systems (including cancer, HIV/AIDS and transplant patients) are particularly vulnerable because their immune systems are not as vigorous as those of healthy adults [7].

Boonmar *et al*. [8] studied a total of 50 and 29 *C. jejuni* isolates from humans and poultry, respectively

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for drug susceptibility and found that all human isolates displayed resistance of 100, 90, 82 and 78% to cephalothin, trimethoprim /sulfamethoxazole, ciprofloxacin and nalidixic acid, respectively. While, poultry isolates showed high levels of resistance to ciprofloxacin and nalidixic acid. Kamberoviæ *et al*. [9] found that all isolates of *C. jejuni* / *coli* isolated from human and poultry meat were resistant to ciprofloxacin and 94.4% of them were erythromycin resistant. Kurin *et al*. [10] declared that macrolides and fluoroquinolones are regarded as drugs of choice for the treatment of human *Campylobacter* infections. The use of antimicrobials for this purpose as well as in food animal production has resulted in the resistance of *Campylobacter* spp. to selected antibiotics.

The aim of the present work was to determine the incidence of *Campylobacter* species and the distribution of antimicrobial resistant campylobacters in food producing animals and human contacts in Egypt.

MATERIALS AND METHODS

Sample Collection: Three hundreds and ten animal samples including 50 fecal (dropping of broiler chickens) and 260 intestinal content (105 broiler chicken, 50 cattle, 55 buffalo and 50 sheep) were collected from El-Monieb and El-warak abattoirs and different animal and poultry farms at Giza Governorate (Egypt) during the period from January 2007 to March 2008. Also, 48 stool samples were collected from persons in contact with investigated food producing animals. The obtained samples were received in sterile screw capped tubes and then transferred immediately to the laboratory in ice box for further investigations.

Bacteriological Examination of the Collected Samples for *Campylobacter*: The collected human and animal samples were inoculated onto charcoal cefoperazone desoxycholate agar (CCDA, Oxoid) medium which is highly selective medium for isolation of *Campylobacter* species. The plates were incubated at 37°C for 72 hours under special microaerophilic condition (85 % nitrogen, 5% oxygen and 10 % carbon dioxide) [11]. The suspected colonies were identified according to the scheme described by Murray *et al*. [11].

Antibiotic Sensitivity Testing of the Isolated *Campylobacter* Strains: The agar disk diffusion technique was adopted according to Quinn *et al*. [12]. The results were interpreted according to the National Committee for Clinical Laboratory Standards [13].

RESULTS

The results are shown in tables 1-3. Bacteriological examination of the collected samples revealed that cattle, buffalo and sheep samples were negative for *Campylobacter* (Table 1). Antibiotic susceptibility testing of *Campylobacter* strains isolated from human and poultry showed multiple antibiotic resistance patterns (Tables 2 and 3).

Table 1: Results of Bacteriological Examination of Animal and Human Samples for *Campylobacter*

Samples	No. of samples	No. of *Campylobacter* isolates	%
Human			
-Stool samples	48	8	16.66 %
Poultry (Broiler chickens)			
-Fecal dropping	50	5	10%
-Intestinal content.	105	12	11.42%
Total	155	17	10.96%
Cattle			
-Intestinal content	50	-	-
Buffalo			
-Intestinal content.	55	-	-
Sheep			
-Intestinal content.	50	-	-

Table 2: Antibiotic Susceptibility of *Campylobacter* Strains Isolated from Human

Human Samples		E10	NA30	S10	C30	TE30	Cip5	CN10	AM10
Total (N=8)	Sensitive	3	6	2	4	2	6	5	1
	%	37.5	75	25	50	25	75	62.5	12.5
	Resistant	5	2	6	4	6	2	3	7
	%	62.5	25	75	50	75	25	37.5	87.5

E10= erythromycin 10 µg, NA30= nalidixic acid 30 µg, S10= streptomycin 10 µg, C30= chloramphenicol 30 µg, TE30= tetracycline 30 µg, Cip5= ciprofloxacin 5 µg, CN10= gentamicin 10 µg, AM10= ampicillin 10 µg, N= number of isolates.

Table 3: Antibiotic Susceptibility of *Campylobacter* Strains Isolated from Poultry

Poultry Samples		E10	NA30	S10	C30	TE30	Cip5	CN10	AM10
Fecal droppings (N=5)	Sensitive	3	4	1	3	3	5	5	2
	%	60	80	20	60	60	100	100	40
	Resistant	2	1	4	2	2	0	0	3
	%	40	20	80	40	40	0	0	60
Intestinal contents (N=12)	Sensitive	4	8	5	3	4	8	7	4
	%	33.3	66.7	41.67	25	33.3	66.7	58.33	33.3
	Resistant	8	4	7	9	8	4	5	8
	%	66.7	33.3	58.33	75	66.7	33.3	41.67	66.7
Total (N=17)	Sensitive	7	12	6	6	7	13	12	6
	%	41.18	70.58	35.29	35.29	41.18	76.47	70.58	35.29
	Resistant	10	5	11	11	10	4	5	11
	%	58.82	29.42	64.71	64.71	58.82	23.53	29.42	64.71

E10= erythromycin 10 µg, NA30= nalidixic acid 30 µg, S10= streptomycin 10 µg, C30= chloramphenicol 30 µg, TE30= tetracycline 30 µg, Cip5= ciprofloxacin 5 µg, CN10= gentamicin 10 µg, AM10= ampicillin 10 µg, N= number of isolates.

DISCUSSION

Increased prevalence of *Campylobacter* in animal production has been associated with farm workers [14]. Farm workers may carry *Campylobacter* from one flock to another if they move between different flocks without changing clothes and boots. Many studies have indicated that the application of hygiene barriers significantly reduced the prevalence of *Campylobacter* in broiler flocks [15].

In the present study, all *Campylobacter* strains were only isolated from poultry (broiler chicken) (10.96%) including 10% from fecal dropping and 11.42% from intestinal content samples. Lower incidence was recorded by Uaboi-Egbennil *et al*. [16] who isolated *Campylobacter* spp. from 9.21% of cloacal samples collected from broiler chicken. Oza *et al*. [17], Baserisalehi *et al*. [18] and Ogden *et al*. [19] recorded prevalence of *Campylobacter* of 67.7, 35 and 41%, respectively from cloacal swabs collected from broiler chicken flocks. Bester and Essack [20] isolated *Campylobacter* from 49.6% of intestinal content samples of broiler and layer chickens.

Bacteriological analysis of the 48 studied human fecal samples showed that 8 (16.66%) were positive for *Campylobacter*. Lower incidences were recorded by Battikhi [21], El-Mohamady *et al*. [22] and Hassanzadeha and Motamedifar [23] as 0.9, 5.6 and 9.6%, respectively. Our results showed that cattle, buffalo and sheep intestinal content samples were negative for *Campylobacter*. Bywater *et al*. [24] found that all intestinal content samples collected from cattle were *Campylobacter* free. Phillips *et al*. [25] isolated *Campylobacter* from sheep carcasses at slaughter facilities at relatively low numbers (less than 1%) after chilling. On the other hand, epidemiological surveys on bovine fecal cultures showed an infection rate ranging from 5 to 50%, with a higher prevalence of *C. jejuni* [26]. Also, many investigators [16, 27] reported 17.5 and 15.3% incidence of *Campylobacter* in sheep, respectively.

In the present work, all the 8 human *Campylobacter* isolates were biochemically identified as *C. jejuni*. This agrees with Hassanzadeha and Motamedifar [23] who found that all the *Campylobacter* strains isolated from human stool were *C. jejuni*. Also, all the 17 poultry *Campylobacter* isolates were biochemically identified as *C. jejuni*. Cardinale *et al*. [28] reported that *C. jejuni* represented 59% of all the campylobacters isolated from chicken carcasses. Oza *et al*. [17] and Bester and Esack [20] studied campylobacters isolated from intestinal samples of broiler chicken and stated that 90.8 and 87%, respectively of the strains were *C. jejuni*.

Resistance among bacteria isolated from food animals 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. The degree of risk posed by this hazard is difficult to estimate, partly because there is a shortage of information regarding the resistance prevalence among bacterial isolates from food producing animals, coupled with inadequate information on both the international distribution and trends of resistance [29, 30]. So, surveillance of the resistance rates among these pathogens of animals and humans is clearly important in risk assessment and management.

Erythromycin is considered the drug of choice in humans for *C. jejuni* infection [2], in our study, most of *Campylobacter* isolates from human (62.5%) were resistant to erythromycin as reported elsewhere [31]. We calculated the resistance rate of *C. jejuni* to erythromycin isolated from broiler chicken and it was (58.82%). Bester and Essack [20] recorded lower rate (50%).

Tetracyclines are relatively inexpensive and have a broad spectrum of activity. For this reason, they have been widely used in the prophylaxis and therapy of human and animal infections and to promote animal growth. These selective pressures have resulted in the emergence of resistant organisms [32]. Gaudreau and Gilbert [33] reported that the rate of resistance of *C. jejuni* to tetracycline rose from 19.1% in 1985-1986 to 55.7% in 1995-1997 which seemed to be increased till now as appeared from our results concerning resistance of *C. jejuni* isolates from human to tetracycline (75%). Our antibiotic susceptibility test results showed that the rate of resistance of *Campylobacter* isolated from broiler chicken to tetracycline was (58.82%). Lower resistance rates were recorded by De Jong *et al*. [30] (41.3%) and Bywater *et al*. [24] (35.4%).

Gaudreau and Glibert [33] stated that the rate of resistance of *C. jejuni* isolated from humans to ciprofloxacin has increased from 3.5% in 1992-1993 to 12.7% in 1995-1997. Resistance to fluoroquinolones (such as ciprofloxacin) in *Campylobacter* isolated from food producing animals is an important emerging public health threat [34]. In the current study, the rate of resistance of *C. jejuni* from humans to ciprofloxacin was 25% which is higher than that reported by CDC [35]. Lower rate (9%) was recorded by Guévremont *et al*. [36]. On the other hand, the rate of sensitivity of *Campylobacter* isolated from broiler chicken to ciprofloxacin was (76.47%). Soonthornchaikul *et al*. [37] reported that all *C. jejuni* isolated from broiler chicken were susceptible to ciprofloxacin.

In the present study, the results of antibiotic susceptibility tests showed that the resistance among *C. jejuni* isolates from broiler chicken to ampicillin was high (64.71%) but higher rate (68%) was reported by Bester and Essack [20] and lower rate (33%) was recorded by Oza *et al*. [17]. In our study, high resistance rate of C. *jejuni* from human to ampicillin was also recorded (87.5%).

Our investigation revealed resistance of the C. *jejuni* strains isolated either from human or broiler chicken to gentamicin and nalidixic acid (37.5 and 29.42%) and (25 and 29.42%), respectively. Oza *et al*. [17] recorded lower resistance rates. In contrary, Abdalameer *et al*. [38] reported that most of *C. jejuni* isolated from human were sensitive to gentamicin and nalidixic acid.

It can be concluded that since *Campylobacter* are zoonotic pathogens, resistance among isolates in animal reservoirs could have consequences for the treatment of infections in animals and humans. So, surveillance of the resistance rates among these pathogens of animals and humans is clearly important in risk assessment and management.

REFERENCES

1. Girard, M.P., D. Steele and C.L. Ghaignat, 2006. A review of vaccine research and development. Human enteric infection. Vaccine, 24: 2732-2750.
2. Nachamkin, I.P., R. Murray, E.J. Baron and J.H. Jorgensen, 2003. *Campylobacter* and *Archobacter*. In: Manual of Clinical Microbiology. Ed 8. Washington, DC: American Society for Microbiology Press, 15: 902-914.
3. Blaser, M.J., B.M. Allos, G.L. Mandell, J.E. Bennett, R. Dolin and M. Douglas, 2005. *Campylobacter jejuni* and Related Species. Bennett's Principles and Practice of Infectious Diseases. Ed 6. Philadelphia: Elsevier Churchill Livingston, 2: 2548-2557.
4. Skirrow, M.B., 1990. *Campylobacter*. Lancet., 36: 921-923.
5. Alfredson, D.A. and V. Korolik, 2007. Antibiotic resistance and resistance mechanisms in *Campylobacter jejuni* and *Campylobacter coli*. FEMS Microbiol. Lett., 277: 123-132.
6. Aarestrup, F.M. and J. Engberg, 2001. Antimicrobial resistance of thermophilic *Campylobacter*. Vet. Res., 32: 311-321.
7. Piddock, L.J., V. Ricci, L. Pumbwe, J.M. Everett and J.D. Griggs, 2003. Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. J. Antimicro. Chemother., 51: 19-26.
8. Boonmar, A., S. Boonmar, L. Sangsuk, K. Suthivarakom, P. Padungtod and Y. Morita, 2005. Serotypes and antimicrobial resistance of *Campylobacter Jejuni* isolated from humans and animals in Thailand. Southeast Asi. J. Trop. Med. Pub. Heal., 36: 531-540. 2005.
9. Kamberoviæ, S., T. Zorman, I. Berce, L. Herman, S.S. Mozina and M. Glas, 2009. Comparison of the frequency and the occurrence of antimicrobial resistance among *C. jejuni* and *C. coli* isolated from human infections, retail poultry meat and poultry in Zenica-Doboj Canton, Bosnia and Herzegovina. Medicinski Glasnik, 6: 147-165.

10. Kurin, M., I. Berce, T. Zorman and S. Mo`ina, 2005. The prevalence of multiple antibiotic resistance in C*ampylobacter* spp. from retail poultry. Meat Food Tech. Biotech., 43: 157-163.
11. Murray, P.R., E.J. Baron, M.A. Pfaller, J.H. Jorgensen, R.H. Yolken and I. Nachmakin, 2003. *Campylobacter* and *Arcobacter* In Manual of Clinical Microbiology. Washington, D C: American Society for Microbiology Press, 5: 902-914.
12. Quinn, P.J., M.E. Carter, B.K. Markey, W.J.C. Donnelly and F.C. Leonard, 2002. Antimicrobial Agents. Great Britain by MPG, Books Ltd, Bodmin, Cornwall. UK. Veterinary Microbiology and Microbial Disease, 7: 28-35.
13. NCCLS (National Committee for Clinical Laboratory Standards), 2002. M-100 Documents: Performance Standards for Antimicrobial Susceptibility Testing, 21: 105-119.
14. Cardinale, E., F. Tall, E.F. Gueye, M. Cisse and G. Salvat, 2004. Risk factors for *Campylobacter spp.* infection in Senegalese broiler-chicken flocks. Prevent. Vet. Med., 64: 15-25.
15. Saleha, A.A., 2004. Epidemiological study on the colonization of chickens with *Campylobacter* in broiler farms in Malaysia: possible risk and management factors. Int. J. Poult. Sci., 3: 129-134.
16. Uaboi-Egbennil, O.P., N.P. Okolie, D.O. Adesanya, E. Omonigbehin and O.A. Sobande, 2008. Epidemiological studies of the incidence of pathogenic *Campylobacter spp.* amongst animals in Lagosmetropolis. Afr. J. Biotech., 7: 2852-2956.
17. Oza, N.A., P.J. McKenna, J.W.S. McDowell, D.F. Menzies and D.S. Neill, 2003. Antimicrobial susceptibility of *Campylobacter* spp. isolated from broiler chickens in Northern Ireland. J. Antimicr. Chemother., 52: 220-223.
18. Baserisalehi, M., N. Bahador and B.P. Kapadnis, 2007. Isolation and characterization of *Campylobacter spp.* From domestic animals and poultry in south of Iran. Pakist. J. Bio. Sci., 10: 1519-1524.
19. Ogden, D.I., F.J. Dallas, M. MacRae, O. Rotariu, W.K. Reay, M.R. Leitch, P.A. Thomson, K.S. Sheppard, M. Maiden, J.K. Forbes and J.C.N. Strachan, 2009. *Campylobacter* Excreted into the Environment by Animal Sources: Prevalence, Concentration Shed and Host Association. Food borne Pathogens and Disease, 6: 1161-1170.
20. Bester, A.L. and Y.S. Essack, 2008. Prevalence of antibiotic resistance in *Campylobacter* isolates from commercial poultry suppliers in KwaZulu-Natal, South Africa. J. Antimicro. Chemother., 62: 1298-1300.
21. Battikhi, M.N., 2002. Epidemiological study on Jordanian patients suffering from diarrhea New microbiology, 25: 405-412.
22. El-Mohamady, H., A.I. Abdel-Messiha, G.F. Youssef, M. Said, H. Farag, I.H. Shsheena, M.D. Rockabranda, B.S. Lubyb, R. Hajjeha, W.J. Sandersa, R.M. Montevillea, D.J. Klenaa and W.R. Frenck, 2006. Enteric pathogens associated with diarrhea in children in Fayoum, Egypt. Diagn. Microbiol. Infect. Dis., 56: 1-5.
23. Hassanzadeha, P. and M. Motamedifar, 2007. Occurrence of *Campylobacter jejuni* in Shiraz, Southwest Iran. Med. Prin. Pract., 16: 59-62.
24. Bywater, R., H. Deluyker, E. Deroover, D.A. Jong, H. Marion, M. McConville, T. Rowan, T. Shryock, D. Shuster, R.V. Thomas, M. Valle and J. Walters, 2004. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. J. Antimicr. Chemother., 54: 744-754.
25. Phillips, D., D. Jordan, S. Morris, I. Jenson and J. Sumner, 2006. Microbiological quality of Australian sheep meat in 2004. Meat Sci. J., 74: 261-266.
26. Wesley, I., S. Wells, K. Harmon, A. Green, L. Schroeder-Tucker, M. Glover and I. Siddique, 2000. Fecal shedding of *Campylobacter* and *Arcanobacter* spp. in dairy cattle. Appl. Environ. Microbiol., 66: 1994-2000.
27. Acik, M.N. and B. Cetinkaya, 2006. Heterogeneity of *Campylobacter jejuni* and *Campylobacter coli* strains from healthy sheep. Veterinary Microbiology, 115: 370-375.
28. Cardinale, E., J. Dromigny, F. Tall, M. Ndiaye, M. Konte and D.J. Perrier-Gros-Claude, 2003. Fluoroquinolone susceptibility of *Campylobacter* strains, Senegal. Emerg. Infect. Dis., 9: 129-134.
29. Franklin, A., J. Acar and F. Anthony, 2000. Antimicrobial resistance: harmonization of national antimicrobial resistance monitoring and surveillance programmes in animals and animal-dervied food. Scientific and Technical Revue, Office International des Epizootiques (O.I.E), 20: 859-870.

30. De Jong, A., R. Bywater, P. Butty, E. Deroover, K. Godinho, U. Klein, H. Marion, S. Simjee, K. Smets, T. Vale´rie, M. Valle´ and A. Wheadon, 2009. A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. J. Antimicr, Chemother., 63: 733-744.
31. Aarestrup, F.M., E.M. Nielsen, M. Madsen and J. Engberg, 1997. Antimicrobial susceptibility patterns of thermophilic *Campylobacter* spp. from humans, pigs, cattle and broilers in Denmark. Antimicr. Agents Chemother., 41: 2244-2250.
32. Chopra, I. and M. Roberts, 2001. Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. Microbiol. Mol. Biol. Rev., 65: 232-260.
33. Gaudreau, C. and H. Gilbert, 1998. Antimicrobial resistance of clinical strains of *Campylobacter jejuni* subsp. *jejuni* isolated from 1985 to 1997 in Quebec, Canada. Antimic. Agents Chemother., 42: 2106-2108.
34. Engberg, J., F.M. Aarestrup, D.E. Taylor, P. Gerner-Smidt and I. Nachamkin, 2001. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli* resistance mechanisms and trends in human isolates. Emerg. Infect. Dis., 7: 24-34.
35. CDC (Centers for Disease Control and Prevention), 1999. Achievements in public health, 1900-1999: Safer and healthier foods. Morbidity and Mortality Weekly Report; 48: 905-913.
36. Guévremont, E., E. Nadeau, M. Sirois and S. Quessy, 2006. Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine and chicken broilers. Can. J. Vet. Res., 70: 81-86.
37. Soonthornchaikul, N., H. Garelick, H. Jones, J. Jacobs, D. Ball and M. Choudhury, 2006. Resistance to three antimicrobial agents of *Campylobacter* isolated from organically-and intensively-reared chickens purchased from retail outlets. The Int. J. Antimicr. Agents, 27: 125-130.
38. Abdulameer, S.M.A., J.M. Sharif, M.N. Khasru and J.A. Rashid 1999. Isolation and identification of *Campylobacter* from diarrheal cases in children of Sulaimany region and the sensitivity tests of the discovered isolates. J. Dohuk Univ., 2: 45-56.