Antimicrobial resistance of Campylobacter jejuni isolated from chicken, some animal products and human in Kalyoubia , Egypt , with special reference to its viability

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A B S T R A C T

Campylobacter species are common bacterial pathogens that cause gastro enteritis in humans, both in industrialized and developing countries.The present study aimed to detect the antimicrobial resistance of Campylobacter jejuni isolated from chicken ,some animal products and human, to detect the viability of C.jejuni in experiementally contaminated yoghurt preserved at 4 °c and to study the effect of 0.5% acetic acid and 4 ml of citric acid on the survival of C.jejuni experiementally inoculated in chicken meat samples. A total of 565 samples were collected . 315 samples from chicken , 122 samples from animal products and 128 human stool swabs. All samples were examined bacteriologically for detection of Campylobacter species using conventional methods. The results revealed that 17.34 % were positive for Campylobacter spp. , 66.32% out of them were identified as C.jejuni . The percentage of antimicrobial resistance of C.jejuni to cephalothin , oxytetracycline, erythromycin , nalidixic acid , ampicillin and gentamicin were 94.7%,63.1%, 52.6% ,36.8% , 21.1% and 5.3% respectively. Also C.jejuni can survive in yoghurt for 7th day after contamination and refrigeration but destructed by addition of 0.5% acetic acid for 30 minutes or 4 ml of citric acid for 1½ hours.

key words : antimicrobial resistance , C.jejuni, viability .

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1. INTRODUCTION

Campylobacter species are world wide major cause of bacterial gastroenteritis (Moore et al.,2005) . C.jejuni and C.coli are responsible for 90% and 10% of human enteric infection cases respectively .(Lastovica ,2006). The main sources of infection by Campylobacter are inadequately cooked meat ,particularly poultry , unpasteurized milk , contaminated drinking water , ready to eat food products, direct contact with animals and fecal runoff of domestic animals and birds contaminating surface water (Whyte et al. ,2004). Campylobacter infections in humans are usually characterized by self limiting watery/bloody diarrhea, abdominal cramps, nausea and fever; however, severe neurological sequelae, bacteremia and other extra intestinal complications may develop infrequently(Blaser and Engberg ,2008).

Campylobacteriosis is often self –limiting and doesn't require antimicrobial treatment .However , in special cases such as septicemia or in the invasive forms of the disease which characterized by sever and prolonged enteritis , as well as in very young patients or immunocompromized individuals , antimicrobial therapy may be needed . Macrolides (erythromycin) and quinolones , including fluoroquinolones (ciprofloxacin , nalidixic acid) are usually used in treatment of Campylobacter infections but in recent years thereis increasing numbers of resistant

Campylobacter isolates , especially to quinolones . Anonymous , 2012)

Because contamination of chicken meat with Campylobacter spp. is un avoidable , there is need for a decontamination step in poultry processing . The treatment of chicken meats with lactic acid or acetic acid, which are classified as generally recognized as a safe , was found advisable for reducing the initial level of C.jejuni and so extending the shelf life of chicken parts by reducing the total microbial load (Cosansu and Ayhan,2008) .The present study aimed to study the antimicrobial resistance of the isolated Campylobacter spp. and to detect the survival of isolated spp. in different environmental conditions.

2. MATERIALS AND METHODS

Collection of samples

Chicken samples : A total of 315 chicken samples were collected; swabs from intestinal contents (105), liver (70) and from muscles (thigh and breast muscles , 70 from each) . Samples were collected from poultry shops at Toukh city , Kalyoubia, Egypt, Animal product samples (52 raw milk samples, 40 kariesh cheese samples and 30 yoghurt samples) were collected from different stores in the same locality and 128 human stool swabs collected from Toukh central hospital .

- Bacteriological examination :

About 10 g of each sample was homogenized in sterile thioglycolate broth. Broth samples were incubated at 42°C for 48 h. Under microaerobic condition (5% O2, 10% CO2 and 85% N2). A loopful of enrichment broth were plated on semisolid thioglycolate broth (Oxoid) and incubated in microaerophilic atmosphere at 25, 37 and 42°C for 48 to 72 h (Gebhart et al. ,1985).The suspected colonies of Campylobacter were identified under phase contrast microscope using (1000×) magnification power to detect their charachteristic motility and morphological charachters (Smibert, 1984). Campylobacter isolates were subcultured for purification and biochemical identification (Frost et al.,1998).

- The antimicrobial sensitivity test:

Commercially prepared disks were used, each of which are pre-impregnated with a standard concentration of a particular antibiotic, for testing their activity against Campylobacter spp. The discs included nalidixic acid (NA) and cephalothin (KF) oxytetracycline (T) , erythromycin (E) , ampicillin (A) and gentamicin (G). (Oxoid Limited,Basingstoke, Hampshire, UK).The antimicrobial susceptibility testing was applied according to the guidelines stipulated by National Committee for Clinical Laboratory Standards "NCCLS" (2001).

Table:(1)Antimicrobial discs, concentration and interpretation of their action on the isolated *Campylobacter* species.

Antimicrobial agent	Sensitivity disc content (ug)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Ampicillin (AM)	10	13 or less	14-17	18 or more
Cephalotin (CN)	30	14 or less	15-17	18 or more
Erythromycin (E)	15	13 or less	14-22	23 or more
Gentamicin (G)	10	12 or less	13-14	15 or more
Nalidixic acid (NA)	30	13 or less	14-18	19 or more
Oxytetracycline (T)	30	14 or less	15-18	19 or more

- *Survival of and viability of C. jejuni*

Preparation of C. jejuni inoculum

C. jejuni isolate was prepared from blood agar plates colonies . A loopful from the

plates was inoculated into thioglycolate enrichment broth and incubated at 42˚C for 48 hours under microaerophilic conditions. After 48 hours, bacterial count of serially diluted broth culture was enumerated using surface plating method (Thatcher and Clark, 1968). After serial dilution of the original broth culture, 100 µl from each dilution was aseptically plated onto mCCDA(modified charcoal cefoperazone deoxycholate agar) plates and incubated at 42˚C for 48 hours under microaerophilic conditions in anaerobic jars (Eideh and Al-Qadiri, 2011). The dilution that had a microbial load of 106CFU/ml was used for the inoculation of yoghurt for detecting viability of C.jejuni and chicken breast meat samples for detecting the effect of organic acids on survival of C.jejuni.(standardization of bacterial count were carried out by "Welcome opacity tubes")

Sampling and sample preparation:

Skinned and deboned chicken breast samples were purchased from a local outlet in Toukh city, Egypt, before conducting the experiment. Each breast meat sample was cut into half . Each piece was then wrapped in aluminum foil and subjected to decontamination and cooking by steaming in a steamer for 3 minutes until core temperature reach 74°c, the internal temperature of chicken breasts was determined by thermometer . steamed chicken breasts were cut into pieces (each piece weighted 10 grams) to provide similar weights for bacterial inoculation and a septically transferred to sterile glass bottles then homogenized in sterile thioglycolate broth and each bottle inoculated with (10) 6 CFU of C.jejuni . The samples were kept for 30 minutes in the bottles to allow enough time for bacterial diffusion into the samples

The inoculated samples were divided into two groups (4 samples each), group I was inoculated with 0.5% acetic acid and group II was inoculated with citric acid (4 ml). Examination of the samples was carried out after half an hour , 1 hr ,1.5hrs and 2 hrs.

3. RESULTS

Occurrence of different Campylobacter species in the examined chicken samples, animal products samples and human. Campylobacter spp. were isolated from chicken samples from 29 intestinal contents swabs (27.6%), 19 liver samples (27.14%) , 5 breast muscles (7.14%) and 10 thigh muscles (14.2%) as shown in table (2) . The isolation rate of C.jejuni was 55.17% from intestinal contents swabs, 68.42% from liver samples, 100% breast muscles and 50% thigh muscles while the isolation rate of C.coli was41.37% from intestinal contents swabs,31.5%from liver samples and 10% from thigh muscles. In addtion to C.lari was isolated from intestinal contents swab(3.4%).T he isolation rate of Campylobacter spp.from animal product samples was 7.4% .11.53% from raw milk, 7.5% from kariesh cheese and 0% from yoghurt.C . jejuni was identified with a percentage of 33.33%, 66.67% and 0% in raw milk samples, kariesh cheese and yoghurt respectively while C.coli was identified with a percentage of 66.67%,33.33% and 0%in raw milk samples, kariesh cheese and yoghurt respectively. In human Campylobacter spp. were isolated from 26 stool swabs(20.3%). C. jejuni were detected in 22 isolates (84.6%) while 4 isolates were identified as C.coli (15.3%). Antimicrobial resistance of Campylobacter spp. In the current study , the antimicrobial susceptibility of isolated C.jejuni were examined and the results revealed that C.jejuni showed resistance against ampicillin (21.1%) ,cephalothin

(94.7%) , oxytetracycline (63.1%) , erythromycin (52.6%) , nalidixic acid (63.8%) and gentamycin (5.3%).Table (3).

The viability of C.jejuni in experimentally contaminated yoghurt :

Our study showed that the inoculated microorganism remain viable for 7 days in the inoculated yoghurt . Table (4).

Effect of 0.5% acetic acid and citric acid (4ml) on the survival of C.jejuni experimentally inoculated in chicken meat samples:

Table (5) showed the results of the effect of using 0.5% acetic acid and citric acid (4 ml) in the survival or viability of C.jejuni which is experimentally inoculated in chicken meat samples . Our results revealed that acetic acid capable of destructing or killing the inoculated C.jejuni in short time (about half an hour) also the results showed that the antibacterial effect of acetic acid is more stronger than that of citric acid which also can destruct the inoculated C.jejuni but within longer period (about 1.5 hour) these results are in agreements with the results recorded by (Berrang et al.,2006 and Birk et al., 2010) .

Table (2): occurrence of different Campylobacter species in the examined chicken samples, animal products and human

Type of samples	Number of examined samples	Positive Campylobacter spp.*	Campylobacter isolates		
			*C.jejuni**	*C.coli**	*C.lari* (NS)
Intestinal contents	105	29 ± 5.3(27.6%)	16 ± 8.8(55.17%)	12± 2.3 (41.37%)	1 ± 2.2(3.44%
Liver	70	19 ± 5.3 (27.14%)	13 ± 8.8 (68.42%)	6± 2.3 (31.57%)	-
Breast muscles	70	5 ± 5.3 (7.14%)	5 ± 8.8 (100%)	-	-
Thigh muscles	70	10± 5.3 (14.2%)	5± 8.8 (50%)	4± 2.3 (40%)	1 ± 2.2 (10%)
Raw milk	52	6± 1.5 (11.53%)	2± 0 (33.33%)	4± 1.5(66.67%)	-
Kariesh cheese	40	3± 1.5(7.5%)	2± 0 (66.67%)	1± 1.5(33.33%)	-
Yoghurt	30	-	-	-	-
Human	128	26± 0.5(20.3)	22± 1(84.6%)	4(15.3%)	-
Total	565	98(17.34%)	65(66.3%)	31(31.6%)	2(2.04%)

Table (3):Antimicrobial resistance of *Campylobacter spp*.

Classes of antibiotics	Antimicrobial agent	S		I		R	
		NO	%	NO	%	NO	%
Cephalosporins (1^{st}) generation	Cephalotin (CN)	1	5.3	-	-	18	94.7
Tetracycline	Oxytetracycline (T)	3	15.8	4	21.1	12	63.1
Macrolids	Erythromycin (E)	3	15.8	6	31.5	10	52.6
Quinolones	Nalidixic acid (NA)	7	36.8	5	26.2	7	36.8
Penicillins	Ampicillin (AM)	12	63.1	3	15.8	4	21.1
Aminoglycosides	Gentamicin (G)	17	89.5	1	5.3	1	5.3

Table (4): The viability of *C.jejuni* in experimentally contaminated yoghurt

Time of culture	Results of isolation
First day:	
1 hour	+ve
2 hours	+ve
3 hours	+ve
Second day	+ve
Third day	+ve
4^{th} , 5^{th} , 6^{th} and 7^{th} days	+ve
8^{th} day	-ve

Table (5): Effect of 0.5% acetic acid and citric acid (4ml) on the survival of *C.jejuni* experimentally inoculated in chicken meat samples

Time	Effect of acetic acid(0.5%)	Effect of citric acid (4ml)
After 30 minutes	100% destruction of *C.jejuni*	100% of *C.jejuni* convert to coccoidfotm
1 hour	100% destruction of *C.jejuni*	100% of *C.jejuni* convert to coccoidfotm
1.5 hours	100% destruction of *C.jejuni*	100% destruction of *C.jejuni*
2 hours	100% destruction of *C.jejuni*	100% destruction of *C.jejuni*

4. DISCUSSION

Campylobacter spp. are important zoonotic infection of significant health hazard due to the relatively low infectious dose , the potentially serious sequelae also the association between certain Campylobacter virulence gene and the pattern of clinical infection . (Al-Mahmeed et al.,2006) . Resistance among Campylobacter spp. represent a potential hazard in that the resistance to the antimicrobial agents reduce the effectiveness of antimicrobial treatment of food borne diseases if contracted by humans (Franklin et al.,2000). Campylobacter resistance to antimicrobial agents has increased during the past decades and has become a matter of concern in sever human Campylobacter infections.(Nachamkin et al.2002) . Higher resistance rates found in developing countries due to the uncontrolled use of antibiotics (Albert,2013) . As mentioned above the results revealed that 17.34 % were positive for Campylobacter spp. , 66.32% out of them were identified as C.jejuni .Table (3) showed that the resistance of C.jejuni strains to ampicillin was at percentage of 21.1% but higher rates recorded by Abd el tawab et al.,2015. Also the current investigation revealed that the resistance of C.jejuni strains to cephalothin was 94.7% . Lower resistance rates recorded by Oza et al.,2003 and khalil et al.,2015 . The resistance of C.jejuni to oxytetracycline , erythromycin and nalidixic acid was 63.1% ,52.6% and36.8%. higher results obtained by Abdeltawab et al.,2015 and Kang et al.2006 ,while lower results recorded by Wasfy et al. ,2000 . Although erythromycin is considered the drug of choice for treatment of Campylobacter infection but it become ineffective due to the increased resistance to this drug in both developed and developing countries (Engberg et al.,2001) .The obtained results in the current study showed 52.6% resistance of C.jejuni to erythromycin . higher results recorded by Abdeltawab et al.,2015, Saad ,2014 , while lower results recorded Wasfy et al. ,2000 . Tetracycline have been chosen to be the alternative drug for the treatment of Campylobacter infection in the past (Trieber and Taylor ,2000). In the current study C.jejuni isolates showed resistance to oxy tetracycline at percentage of 63.1% . Higher results obtained by Bester and Essack ,2012 and Kang et al.,2006. The high antimicrobial resistance rate to tetracycline may be due to their use in veterinary medicine for prevention and control of poultry diseases (Harriharan et al.,2009) . Gentamicin is one of the aminoglycosides widely used for treatment of systemic Campylobacteriosis infections (Skirrow and Blaser ,2000). The results in the present study revealed that 5.3% of C.jejuni isolates were resistant to gentamicin .Nearly similar results obtained by Bester and Essack ,2012. While higher resistance rates recorded by Abdeltawab et al.,2015.The low level of resistance to gentamicin may be attributed to the fact that gentamicin is rarely used in poultry

industry either as a prophylactic or for treatment as it given by intramuscular route which make it impracticable for large scale application on poultry farms (Rahimi and Ameri,2011) . Studying the viability of isolated strain of C.jejuni in yoghurt is determined through experiemental inoculation of yoghurt with (10)6 cfu of C.jejuni . The current study showed that the inoculated microorganism remain viable for 7 days in the inoculated yoghurt , table (4) . These results are comparable with the results recorded by Barakat et al.,2015.

The in activation of C.jejuni in acidified food hasn't been well studied yet. Although their counts were reduced after exposure to 1% lactic acid for 5 minutes in broth at low temperature. As well as the effect of organic acids on Campylobacter survival depends on either the testing is performed in broth or in food matrix , as the food matrix leads to decreasing the accessibility of the bacteria so limiting the effect of lactic acid on killing the bacteria and make its use as a decontaminant in the food processing is rejected by the European food safety authority (Stern et al.,1985) . Table (5) showed the results of the effect of using 0.5% acetic acid and citric acid (4 ml) in the survival or viability of C.jejuni which is experimentally inoculated in chicken meat samples . Our results revealed that acetic acid capable of destructing or killing the inoculated C.jejuni in short time (about half an hour) also the results showed that the antibacterial effect of acetic acid is more stronger than that of citric acid which also can destruct the inoculated C.jejuni but within longer period (about 1.5 hour) these results are in agreements with the results recorded by (Berrang et al.,2006 and Birk et al., 2010) . The acidic ingredients either used alone or in combination were effective for reduction of C.jejuni population . In both broth and chicken juice ,0.5% concentration of the organic acids was efficient in reduction of C.jejuni population. Therefore marination of broiler meat could be used as an intervention to control Campylobacter species (Birk et al.,2010). Finally , it can be concluded that the presence of high level of multi drug resistance of Campylobacter isolates was reported and the possible cause of these resistance could be the wide spread use of antibiotics in chicken and cattle farms. So it is recommended that in vitro, antimicrobial susceptibility testing of Campylobacter should be performed for obtaining good results in treatment especially for those cases of food borne Campylobacteriosis with sever or prolonged symptoms or in immune-compromized patients.0.5% acetic acid and citric acid are valuable ingredients in destructing C.jejuni in poultry meat .Reduction of infection on the animal farms and control of Campylobacter infection in poultry would reduce the risk of human exposure to Campylobacter and decrease the prevalence of infection .

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