

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

***Helicobacter pullorum* among Poultry in Assiut-Egypt: Genetic Characterization, Virulence and MIC**

Moemen A. Mohamed, Ragab S. Ibrahim, Moustafa A. Shahata and El-Refaie M. El-Refaie
Department of Poultry Diseases, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

Abstract: The molecular characterization, the MICs of antibiotics (with clinical relevance in human and veterinary medicine) for the selected isolates of *H. pullorum* and its Pathogenicity to chickens were carried out. A total of 1800 samples (cloacal swabs, cecal contents and liver) were collected from chickens, turkeys and ducks. *H. pullorum* could be identified only from chickens with a percentage 39.33%. One hundred isolates were identified as *H. pullorum* by phenotypic tests. Those were subjected to Polymerase Chain Reaction (PCR) assay based on 16S rRNA to confirm phenotypic identification. These results revealed 100% accordance between phenotypic and genotypic identification and a considerably shortened analysis time, provides a sensitive and rapid alternative for applications in the identification of *H. pullorum* and highlights the potential of PCR technology in routine pathogens identification. The prevalence of *cdtB* gene was analyzed among the examined *H. pullorum* strains (n = 100) using PCR assay. The *cdtB* gene was present in nearly from 79 *H. pullorum* strains out of 100 examined ones. Fifty-three out of the 79 *cdtB* positive strains (67%) were obtained from diseased birds. In contrast to 26 out of 79 positive *cdtB* gene strains (32.9%) could be obtained from clinically healthy chickens. This different distribution of genetic virulence markers between isolates may indicate that gene may play a role in the pathogenesis of this organism to chickens. Infection was induced in 39 healthy one-day-old chicks with isolated *H. pullorum* strain by oral route. The mean incubation time was 96 h. Considerable weight loss was observed in the experimental group compared with the control group. Diarrhea observed in chicks from the 5th day post infection. Postmortem revealed unabsorbed yolk sac and distended ceca. The susceptibilities of *Helicobacter pullorum* (20 strains) to 6 antimicrobial agents were investigated by determination of the MIC using the Micr-broth dilution method. All examined strains were resistant to ciprofloxacin, gentamicin and erythromycin followed by tetracycline (70%) and complete susceptibility to colistin and ampicillin, indicating the possibility of chicken being a source of the resistance tool for humans.

Key words: Antibiotics, veterinary medicine, pathogens identification

INTRODUCTION

The genus *Helicobacter*, belonging to the class Epsilonproteobacteria was established in 1989 and comprises from 28 species (Wikipedia) of microaerobic, Gram-negative, curved, spiral and nonsheathed flagellated bacteria. *Helicobacter pullorum*, is a urease-negative organism was classified as a new species of *Helicobacter* by Stanley *et al.* (1994). This organism has been found in the livers and intestinal contents of laying hens with vibronic hepatitis and from the caeca of broiler chickens (Stanley *et al.*, 1994; Atabay *et al.*, 1998). Also, *H. pullorum* is an enterohepatic pathogen contribute to human disease, as suggested by reports of its isolation from feces and biopsies of patients with gastroenteritis, chronic liver disease and inflammatory bowel disease (Ceelen *et al.*, 2005).

Avian species serve as reservoirs for the *Helicobacter pullorum* (Andersen, 2001). The true prevalence of *H. pullorum* in poultry may be underestimated due to misidentification, since this species shares many phenotypic similarities with *Campylobacter* species and accurate tests to distinguish among them are lacking

(Atabay *et al.*, 1998). As well as the fastidious growth requirements of *H. pullorum* for selective recovery and detection of the bacterium in poultry is known to be a difficult work (On, 2001).

As a consequence, studies on the prevalence of *H. pullorum* in poultry are limited (Burnens *et al.*, 1996; Atabay *et al.*, 1998; Ceelen *et al.*, 2006a). To date, there have been no formal reports on the presence of *H. pullorum* in chickens in Assiut province and Upper Egypt, so the aims of this study were to investigate the occurrence of *H. pullorum* using the cultural and molecular methods among poultry flocks.

In addition, as there is little information about *H. pullorum* antibiotic resistance and genetic makeup (Ceelen *et al.*, 2006a,b), an objective of this study was also to investigate the antibiogram patterns of the isolates to different antimicrobial agents using the agar dilution method and the extent of the cytolethal distending gene (*cdt*) presence among isolated *H. pullorum* strains as a putative virulence marker for *H. pullorum* due to its role in hijacking the control system of eukaryotic cells that results in G2 arrest in the cells and

induces apoptotic cell death of lymphocytes, which may be relevant to onset or persistence of chronic infection by the producing bacteria (Ohara *et al.*, 2004).

The interactions of *H. pullorum* with its natural host have not yet been studied. To obtain better insights in the pathogenesis of *H. pullorum* infections in poultry, an experimental infection model was developed in broilers and the colonization capacity and preferred colonization site of different *H. pullorum* strains isolated from poultry and humans was determined.

MATERIALS AND METHODS

Sampling, isolation and identification: One thousand eight hundreds samples were taken from cloacal swabs, cecum and liver that were collected from chickens, turkeys and ducks from different regions in Assiut province.

Samples were inoculated into Brain Heart Infusion (BHI) broth containing 10% sterile inactivated horse serum and Skirrow's supplement then incubated in microaerophilic condition (5% H₂, 5% CO₂, 5% O₂ and 85% N₂) in CampyPak II anaerobic system jar with CampyPak gas generating system for 48 hours. Sub-culturing was carried out on (BHI) agar plates enriched with 5-10% sheep blood and containing Skirrow's supplement and incubate cultured plates at 37-42°C for 72 h under microaerophilic atmosphere. The growth was examined for typical *Helicobacter pullorum* colonies.

The colonial form and phenotypic characteristics (gram-negative, slightly curved rod, catalase and oxidase positive and indoxyl acetate negative) of the isolates were used for presumptive identification.

Species confirmation using PCR: Total genomic DNA extraction was performed with QIAamp DNA mini extraction kit (Qiagen, Germany) according to the recommendations of the manufacturer. The DNA concentration was determined by UV spectrophotometer (Beckman DU 640, CA, USA) and adjusted to 50 ng/μl. Three microlitres (150 ng) of each template was used for the PCR. Species identity was confirmed using the *H. pullorum* species-specific 16S rRNA gene PCR assay (Stanley *et al.*, 1994). In brief, the primer sequences were: 5-ATGAAT GCT AGT TGT TGT CAG-3 (forward) and 5-GATTGG CTC CAC TTC ACA-3 (reverse) (Bioneer incorporation Daejaon 306-220, Korea) and the cycling conditions involved an initial denaturation of 95°C for 1 min, followed by 30 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1 min 15 s. The 448 bp product was detected by ethidium bromide staining as described previously (Stanley *et al.*, 1994).

Detection of *cdtB* gene using PCR: A *cdtB* was detected by PCR amplification using the following primers; forward (5-TCA GCT AGC ACA GAA AAT AAA TGG-3) and reverse (5-AGT AAT TTG TTG CAT ATT CAT AAA-3) were

synthesized at Bioneer incorporation Daejaon 306-220, Korea and designed in this study depending on the gene sequence on pubmed (AF123536) to target a 780-bp fragment of the *H. pullorum cdtB* gene.

PCR reaction was performed in a 50 μl reaction mix containing: Twenty five μl of 2X GoTaq Green Master Mix (Promega, USA), one μl of 500 pM forward primer, one μl of 500 pM reverse primer, two μl of undiluted template DNA containing 50 ng DNA/μl and twenty one μl of DNase and RNase free water.

The parameters for all reactions were summarized in the following profile, initial denaturation at 94°C for 5 min then 35 cycles of 94°C for 1 min for denaturation, annealing 50°C for 1 min and extension for 3 min at 72°C. The final extension took 10 min at 72°C. A 10 ul sample of the assay was electrophoresed through a 2% agarose gel containing Tris-Acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 2 mM EDTA, pH 7.5) and ethidium bromide (0.5 mg/ml) in TAE buffer at 5.5 V/cm for 1 h. The gel was viewed by ultraviolet transillumination.

Experimental infection: Fifty two clinically healthy one-day-old Ross breed broiler chickens were randomly divided into two experimental groups: Infected (n = 39) and untreated control (n = 13).

The isolated *H. pullorum* strain was used for inoculum preparation, grown on BHI broth (Oxoid, Drogen, Belgium) and agar, supplemented with 10% horse blood. Incubation was done under microaerobic conditions (5% H₂, 5%CO₂, 5%O₂ and 85% N₂) at 37°C for 72 h. *H. pullorum* cultures were harvested by washing the plates with Phosphate-Buffered Saline (PBS) followed by centrifugation (1500 x g, 10 min, 4°C). The pellet was subsequently resuspended in PBS to an Optical Density (OD) of 0.7 at 600 nm which corresponds to approximately 8 log₁₀ CFU/ml. All chicks of group A were inoculated with one of the *H. pullorum* strains at one day of age. Two hundred μl of bacterial inoculum containing 7 log₁₀ CFU was given to each chicken by gavage. Group B was maintained as a control group and received 200 μl PBS. All birds were clinically examined on a daily basis.

Antimicrobial susceptibility testing: Susceptibility testing was performed by a broth microdilution method in microtiter tray as described by (Jennifer, 2001). Six antimicrobial agents were tested: ciprofloxacin, tetracycline, ampicillin, colistin, gentamicin and erythromycin. All antimicrobial agents were purchased from Sigma, except for ciprofloxacin which was obtained from Bayer AG (Leverkusen, Germany). The antibiotic concentrations ranged from 0.25-256 μg/ml¹.

Since no breakpoints are currently available for *H. pullorum*, we tentatively used Enterobacteriaceae breakpoints as described by the Clinical and Laboratory Standard Institute (CLSI, formerly NCCLS) for *Campylobacter jejuni* and related species (NCCLS, 2002).

Table 1: The positive percentage of examined samples according to its type

Bird status	Sample type			Total/percentage of infection
	Cloacal swabs	Liver	Cecal samples	
Healthy birds	195 (10) ^a	195 (84)	195 (120)	585 (214) 36.6%
Diseased birds	105 (11)	105 (57)	105 (72)	315 (140) 44.4%
Total	300 (21)	300 (141)	300 (192)	900 (354) 39.3%

^aNumber in brackets is the total number of positive samples examined

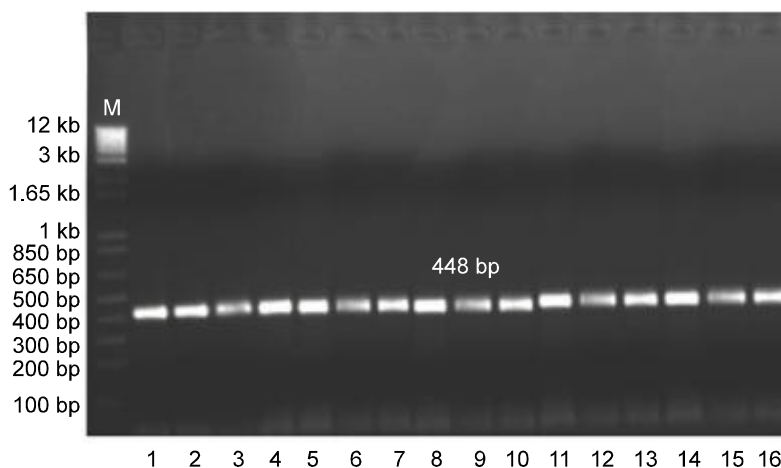


Fig. 1: *Helicobacter pullorum* positive samples using PCR with the species-specific primer set to 16S rRNA gene of *H. pullorum*

RESULTS

As shown in Table 1, the highest isolation rate was achieved with cecal samples followed by liver samples then cloacal swabs as 64% (192 out of 300), 47% (141 out of 300) and 7% (21 out of 300), respectively with overall percentage 39.33% (354 out of 900). *Helicobacter* suspected colonies were observed among 354 of 900 samples (39.33%). It was observed that the higher incidence rate obtained from samples collected from birds suffer from enteritis than that collected from apparently healthy birds.

Helicobacter pullorum specificity was examined using PCR with the species-specific primer set designated as previously described by Stanley *et al.* (1994). One hundred of the original 354 isolated *H. pullorum* isolates were subjected to 16S rRNA gene amplification as shown in Fig. 1. According to the obtained results, all examined strains were positive for *H. pullorum*.

Identification of *cdtB* gene in *H. pullorum* isolates: The designed primers which are based on the gene sequence on pubmed with accession number (AF123536) produced PCR amplicons of the expected sizes 780 bp from 79 *H. pullorum* strains out of 100 examined ones as shown in Fig. 2. Fifty-three out of the 79 *cdtB* positive strains (67%) were obtained from diseased birds. In contrast to 26 out of 79 positive *cdtB*

gene strains (32.9%) could be obtained from clinically healthy chickens.

Experimental infection: A total 13 out of 39 infected chicks were died starting from 4th day post infection as shown in Table 2. Died chicks at 4th day post infection did not show any clinical signs while signs of loss of appetite, depression, ruffled feathers, anorexia and yellowish white diarrhea were observed in chicks died from 5th day post infection. Post-mortem examination of dead chicks revealed distended abdomen, unabsorbed yolk sac with sever congestion and dark yellow to brown contents, distended ceca with frothy yellowish exudates, mild fibrinous pericarditis and air-sacculitis, in some cases hydropericarium, might be noticed and in some cases yellowish gelatinous exudates might be present in abdominal cavity. Survivors were retarded in growth with poor conversion rate. They expressed symptoms of weakness, depression, loss of appetite, diarrhea and mild respiratory signs.

Antimicrobial susceptibility: The MIC values of the twenty isolates tested are shown in Table 3. The results reveled that all examined strains were resistant to ciprofloxacin, gentamicin and erythromycin, while 14 out of 20 isolates resistant to tetracycline and All isolates were susceptible to colistin and ampicillin. Monomodal courses for the MICs were found for ampicillin.

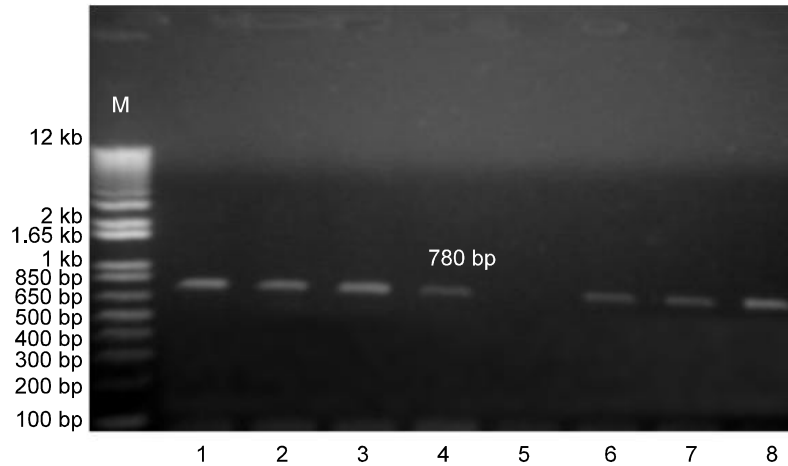


Fig. 2: PCR amplicon of CdtB gene among *H. pullorum* strains

Table 2: Results of experimental infection of one-day old chicks with *Helicobacter pullorum* isolates

Group	Inoculated agent	No. of birds	Rout of inoculation	No. of deaths/day post infection						TND	NS	MP
				4th day	5th day	6th day	7th day	8th day	9th day			
Experimental	<i>H. pullorum</i> isolates	39	Oral	4	3	2	2	1	1	13	26	33.3%
Control	Sterile BHI broth	13	Oral	-	-	-	-	-	-	-	13	00.0%

TND = Total No. of Deaths; NS = No. of Survivors; MP = Mortality Percentage

Table 3: Distribution of MICs for 20 chicken isolates of *Helicobacter pullorum* to antimicrobial agents

Antibiotic	No. of isolates with MIC (µg/mL) of:											BDR	NRI	RS (%)
	0.25	0.5	1	2	4	8	16	32	64	128	256			
Ciprofloxacin	-	-	-	-	-	7	5	8	-	-	-	≥4	20	100
Gentamicin	-	-	-	-	-	-	-	12	8	-	-	≥16	20	100
Colistin	-	-	-	-	-	-	10	10	-	-	-	≥32	-	0
Tetracycline	-	-	-	-	-	-	6	11	3	-	-	≥16	14	70
Erythromycin	-	-	-	-	-	-	8	5	7	-	-	≥8	20	100
Ampicillin	-	-	-	-	-	7	-	13	-	-	-	≥32	-	0

BDR = Break-point of Drug Resistance; NRI = No. of Resistant Isolates; RS = % of resistant isolates

DISCUSSION

The present study shows a moderate prevalence of *H. pullorum* in Chickens 39.3% with the highest isolation rate was achieved with caecal droppings while the lowest success was with cloacal swabs or faecal droppings. In contrast to the incidence rate among turkeys and ducks was zero percentage. Comparing our study results to those obtained by Atabay *et al.*, 1998, the latter found a higher occurrence of *H. pullorum* (60%) on poultry carcasses. This clear difference in isolation rate could be due to cross-contamination with cecal contents on the surface of broiler carcasses during poultry processing (Stanley *et al.*, 1994). On reverse Burnens *et al.* (1994), found a prevalence rate of 4% upon sampling cecal contents of broilers. Considering the fastidious nature of this organism, this finding could explain this markedly lower percentage of positive birds.

Regarding the highest isolation rate from cecum, Celeen *et al.* (2006a) assumed that the lower segments of the intestinal tract are the predominant colonization sites for *H. pullorum* in broiler chickens. *H. pullorum* may gain access to the liver by retrograde movement from the duodenum. Alternatively, it may transfer from the gut lumen to the portal circulation.

The confident identification of *H. pullorum* is necessary to avoid its complication with *Campylobacter* spp. that share a common habitat within the caecum and large intestine of chickens (Corry and Atabay, 2001). As an alternative approach, PCR-based methods have been used as developed by Stanley *et al.* (1994) to confirm identification of all field isolates of *H. pullorum*. The results of this study have shown that all the preliminary identified *H. pullorum* were positive 16S rRNA that specific for this species by PCR. So, a combination of

genotypic and phenotypic methods may be useful in the identification of *H. pullorum* organisms.

Our results indicate that there was no incidence of *Helicobacter pullorum* in poultry species other than chickens like turkeys and ducks (living and/or dead); this might be due to genetic resistance of these poultry species to *H. pullorum* organism.

Helicobacter pullorum is a bacterium with zoonotic potential (Young *et al.*, 2000a, b; Pellicano *et al.*, 2004; Ceelen *et al.*, 2005). Similar to the closely related genera *Campylobacter* and *Arcobacter*, it is assumed that poultry may be the vehicle for human infections. The results of the present study exhibit that 79 *H. pullorum* strains out of 100 examined ones harbored *cdtB* gene. Fifty-three out of the 79 *cdtB* positive strains (67%) were obtained from diseased birds. In contrast to 26 out of 79 positive *cdtB* gene strains (32.9%) could be obtained from clinically healthy chickens, suggesting that toxin gene could play a role in the pathogenesis of this microorganism in chickens, the production of this toxin is believed to play a role in producing diarrhoea in human patients infected with *Helicobacter pullorum* (Chien *et al.*, 2000).

The existence of other virulence markers including the production of other toxins and the phenomenon of adhesion and colonization capacity of *Helicobacter pullorum* need to be examined to unlock more of the secrets of the lifestyle of this emerging pathogen (*Helicobacter pullorum*) (Ceelen *et al.*, 2006a,b).

In the experiment presented here, the intestines from experimentally *H. pullorum*-infected chickens were macroscopically examined and the results revealed the presence of distended caeca with foamy contents in most of the *H. pullorum*-infected chickens in agreement with that previously reported by (Ceelen *et al.*, 2007). The findings in this study question the statement as to *C. jejuni* being solely responsible for these marked necropsy findings as mentioned by (Clark and Bueschkens, 1988).

In the current experiment, no macroscopic lesions were present in the liver samples of infected chicks. In disagreement with our findings Stanley *et al.* (1994) and Burnens *et al.* (1996) noticed a vibronic hepatitis in *H. pullorum* infected laying hens that characterized by swelling and necrosis of the liver (Berry and Whitenack, 1991). This difference in results might be explained by the young age of the infected birds. Similar results have been noticed with *H. hepaticus*-infected mice where hepatic lesions only start to develop at an older age depending on mouse strain (Li *et al.*, 1998; Whary *et al.*, 1998). The results of this experiment demonstrate first step in the demonstration of the interaction of *H. pullorum* with chicken that throw a demand for a further research.

Based on NCCLS (2002) breakpoints for *Campylobacter*, a high level of resistance (100%) was observed for 3 antibiotics: ciprofloxacin, gentamicin and

erythromycin and 70% of examined strains were resistance to tetracycline. In view of the whole range of antibiotics available in Egypt and the lack of legislative restrictions on their use for therapy, prophylaxis, or growth promotion, so the high incidence of antibiotic resistance observed in the present study is not surprising. However, resistance to ampicillin and colistin was zero, reflecting the infrequent use of this antibiotic in poultry breeding in Assiut. We can state that ampicillin and/or colistin sulfate are the drugs of choice that can help in prevention and control of *Helicobacter pullorum* infection in chickens. This first study should constitute a basic reference for further surveys of antibiotic-resistance of *H. pullorum* isolates in Assiut-Egypt. The evolution of antibiotic resistance of avian *H. pullorum*, together with the evolution of therapeutic practices, should be controlled by a network of epidemiological survey of poultry breeding in Egypt.

REFERENCES

- Andersen, L.P., 2001. New *Helicobacter* species in humans. Dig. Dis., 19: 112-115.
- Atabay, H.I., J.E. Corry and S.L.W. On, 1998. Identification of unusual *Campylobacter*-like isolates from poultry products as *Helicobacter pullorum*. J. Applied Microbiol., 84: 1017-1024.
- Berry, J.G. and D. Whitenack, 1991. Bacterial diseases of poultry. Excluding respiratory diseases, Oklahoma Cooperative Extension Service F-9109: pp: 1-4.
- Burnens, A.P., J. Stanley, R. Morgenstern and J. Nicolet, 1994. Gastroenteritis associated with *Helicobacter pullorum*. Lancet, 344: 1569-1570.
- Burnens, A.P., J. Stanley and J. Nicolet, 1996. Possible association of *Helicobacter pullorum* with lesions of vibronic hepatitis in poultry. In: *Campylobacters, helicobacters and related organisms*, Newell D.G., Ketley J.M. and Feldman R.A. (Ed.), New York: Plenum Press, pp: 291-293.
- Ceelen, L.M., A. Decostere, G. Verschraegen, R. Ducatelle and F. Haesebrouck, 2005. Prevalence of *Helicobacter pullorum* among patients with gastrointestinal disease and clinically healthy persons. J. Clin. Microbiol., 43: 2984-2986.
- Ceelen, L.M., A. Decostere, K. Van den Bulck, S.L. On, M. Baele, R. Ducatelle and F. Haesebrouck, 2006a. *Helicobacter pullorum* in chickens, Belgium. Emerging Infect. Dis., 12: 263-267.
- Ceelen, L.M., F. Haesebrouck, H. Favoreel, R. Ducatelle and A. Decostere, 2006b. The cytolethal distending toxin among *Helicobacter pullorum* strains from human and poultry. Vet. Microbiol., 113: 45-53.
- Ceelen, L.M., A. Decostere, K. Chiers, R. Ducatelle, D. Maes and F. Haesebrouck, 2007. Pathogenesis of *Helicobacter pullorum* infections in broilers. Int. J. Food Microbiol., 116: 207-213.

- Chien, E.Y., C.A. Dangler, J.G. Fox and D.B. Schauer, 2000. *Helicobacter hepaticus* infection triggers inflammatory bowel disease in T cell receptor alphabeta mutant mice. *Comp. Med.*, 50: 586-594.
- Clark, A.G. and D.H. Bueschkens, 1988. Horizontal spread of human and poultry-derived strains of *Campylobacter jejuni* among broiler chickens held in incubators and shipping boxes. *J. Food Prot.*, 51: 438-441.
- Corry, J.E. and H.I. Atabay, 2001. Poultry as a source of *Campylobacter* and related organisms. *Symp. Ser. Soc. Appl. Microbiol.*, pp: 96S-114S.
- Jennifer Andrews, M., 2001. Determination of Minimum Inhibitory Concentrations. *J. Antimicrobial Chemotherapy*, 48: sup S1, 5-16.
- Li, X., J.G. Fox, M.T. Whary, L. Yan, B. Shames and Z. Zhao, 1998. SCID/NCr mice naturally infected with *Helicobacter hepaticus* develop progressive hepatitis, proliferative typhlitis and colitis. *Infect. Immunity*, 66: 5477-5484.
- National Committee for Clinical Laboratory Standards (NCCLS), 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, Second edition. NCCLS, Wayne, PA, Approved Standard M31-A2, pp: 80.
- Ohara, M., T. Hayashi, Y. Kusunoki, M. Miyauchi, T. Takata and M. Sugai, 2004. Caspase-2 and caspase-7 are involved in cytolethal distending toxin-induced apoptosis in Jurkat and MOLT-4 T-cell lines. *Infect. Immunity*, 72: 871-879.
- On, S.L.W., 2001. Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: Current status, future prospect and immediate concerns. *Symp. Ser. Soc. Appl. Microbiol.*, pp: 1s-15s.
- Pellicano, R., V. Mazzaferro, W.F. Grigioni, M.A. Cutufia, S. Fagoonee, L. Silengo, M. Pizzetto and A. Ponzetto, 2004. *Helicobacter species* sequences in liver samples from patients with and without hepatocellular carcinoma. *World J. Gastroenterol.*, 10: 598-601.
- Stanley, L.D., A.P. Burnens, F.E. Dewhirst, S.L.W. On, A. Porter, R.J. Owen and M. Costas, 1994. *Helicobacter pullorum sp. nov.* - genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. *Microbiology*, 140: 3441-3449.
- Whary, M.T., T.J. Morgan, C.A. Dangler, K.J. Gaudes, N.S. Taylor and J.G. Fox, 1998. Chronic active hepatitis induced by *Helicobacter hepaticus* in the A/JCr mouse is associated with a Th1 cell-mediated immune response. *Infect. Immunity*, 66: 3142-3148.
- Wikipedia: <http://en.wikipedia.org/wiki/Helicobacter>.
- Young, V.B., K.A. Knox and D.B. Schauer, 2000a. Cytolethal distending toxin sequence and activity in the enterohepatic pathogen *Helicobacter hepaticus*. *Infect. Immunity*, 68: 184-191.
- Young, V.B., C.C. Chien, K.A. Knox, N.S. Taylor, D.B. Schauer and J.G. Fox, 2000b. Cytolethal distending toxin in avian and human isolates of *Helicobacter pullorum*. *J. Infect. Dis.*, 182: 620-623.