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## CONCLUSION AND RECOMMENDATION

Production of milk and milk products is subjected to many risks of contamination due to absence of pasteurization, bad quality ingredients and unsanitary methods of processing and distribution. Therefore, production of safe and high quality foods with a minimum of contamination ranks a prime consideration of food hygienists.

The results of this work emphasized that raw milk and some milk products (ice-cream and Kareish cheese) available for consumption in Sohag City were contaminated by some *Helicobacter* species including *H. cinaedi*, *H. felis*, *H. hepaticus*, *H. mustelae*, *H. pullorum* and *H. pylori*, this may reflect the lack of hygienic supervision, poorly sanitized equipments, which probably constitute the major sources of contamination of milk and milk products.

Although the contamination of raw milk, ice-cream and Kareish cheese with *H. pylori* in this study is extremely small it should not be ignored and it is important to recognize their presence which may result in potential health hazards. Furthermore, the presence of any species of *Helicobacter* is indicative of the potential presence of *H. pylori* and should be taken very seriously because the physiology and habitat of the different species of *Helicobacters* are very similar.

Comparison between HPSPA and Columbia agar postulated that the use of one medium was not enough for isolation of *Helicobacters*. It was recommended that HPSPA and Columbia agar media and their

antibiotic supplement were the most selective and differential media for isolation of *Helicobacter* spp.

From the achieved results it is evident that plasmid DNA could be detected in all *H. pylori* isolates. The plasmid occurrence rate was high (100%) and of high molecular weight with resistance to amoxicillin, metronidazole and nalidixic acid. The antibiogram of *H. pylori* isolated from raw milk and ice-cream revealed high sensitivity to cephalothin and tetracycline and moderate sensitivity to clarithromycin, augmentin and unasyn.

Development of drug resistance is one of the most serious complications of chemotherapy. The emergence of resistant mutant is encouraged by inadequate dosage, prolonged treatment, the presence of a closed focus of infection and the abuse of antibiotics without in vitro susceptibility testing. So, periodic surveillance of antibiotic resistance testing for both pathogenic and non pathogenic bacteria in humans, live stocks, food stuffs and environment are used to detect the emergence of these resistant genes among bacterial species.

Honey has been gaining interest as a substitute sweetener in foods such as milk due to its healthy and natural image and its inhibitory properties against pathogens. The data obtained showed significant effect of honey on survival of *H. pylori* in milk. So it is likely to sweeten the milk especially for children with honey instead of other sugars.

Therefore, the aforementioned data proved that we must pay great attention to the problems of these pathogens in our foods. Consequently, more restriction and preventive measures should be taken to improve the

quality of raw milk and milk products to protect consumers from being infected by *Helicobacters* which require many precautions including :

- High quality milk produced under the most possible hygienic conditions should be used.
- Proper cleaning and sanitizing of all dairy utensils and equipments used on the farm or in the plant.
- Good hygienic precautions should be taken with house hold pets to prevent infection via this source.
- Drinking water and water used in food industry should be properly treated (chlorinated) to inactivate the pathogens if present.
- Handling and distribution of milk and milk products should be done under strict hygienic measures.
- Elimination of flies, rodents and other insects from food establishment is essential.
- Educational programs for farmers, consumers and plant employees are also indispensable. There are two mains by which attempts have been made to obtain a safe food supply : a-Inspection and regulation, b-End product testing.
- Avoid consuming raw milk, the public should be made aware of the hazard of Helicobacters and other pathogens in raw milk and should be advised to consume pasteurized milk and milk products as Helicobacters will not survive the efficient pasteurization process for milk and milk products.
- The use of refrigeration during processing, storage and handling of the dairy product is also essential.
- Using honey as a substitute sweetner in foods due to its inhibitory properties against *H. pylori*.

- The HACCP should be designed to establish environment-monitoring programs to minimize the potential for contamination of finished products and to identify contaminated foods before distribution.
- Application of the Good Manufacturing Practices (GMPs) in milk and milk products, manufacturing units should be regarded by the regulatory authorities as basic or compulsory requirements.
- Strict application of the “General Principles of Food Hygiene” issued by the “Codex Alimentarius Commission” and should be practised in all chain of milk and milk products manufacturing.

## SUMMARY

A total of 330 random samples of milk and some milk products including raw milk (150), soft cheeses (Damietta and Kareish cheese), ice cream (50 samples each) and cooking butter (30) samples, were collected from street vendors, farmer's houses, dairy farms and dairy shops in Sohag City. These samples were examined for the prevalence of *Helicobacter* species using two selective media: Helicobacter pylori special peptone agar (HPSPA) and Columbia blood agar base.

The results revealed that 29 (19.3%), 10 (20%), 15 (30%) of the examined raw milk, Kareish cheese and ice-cream samples were contaminated with *Helicobacter spp.* on HPSPA. However, the incidence of *Helicobacter spp.* on Columbia agar was 22 (14.7%), 3 (6%), 13 (26%) in the same samples, respectively.

*H. pylori* was isolated in a percentage of 0.7% and 2% from the examined raw milk and ice-cream samples, respectively on HPSPA and in a percentage of 1.3% from the examined raw milk samples on Columbia agar.

In addition, other *Helicobacter spp.* were isolated from the examined samples as *H. cinaedi* which recovered in percentages of 6.7, 6 and 8% from the examined raw milk, kareish cheese and ice-cream samples, respectively on HPSPA, while on Columbia agar its incidence was 2, 6 and 6% from the same examined samples. While, *H. pullorum* was also isolated in a percentage of 8, 6 and 12% on HPSPA from the examined raw milk, karesih cheese and ice-cream samples, while it was

5.3 and 12% from the examined raw milk and ice-cream samples on Columbia agar. *H. hepaticus* was recovered from examined raw milk, kariesh cheese and ice-cream samples in percentages of 3.3, 6 and 4% on HPSPA while recovered in 4.7 and 6% from the examined raw milk and ice-cream samples on Columbia agar. On the other hand, *H. felis* was isolated in percentages of 6.7, 2 and 2% from the examined raw milk, kariesh cheese and ice-cream samples on HPSPA while, on Columbia it was recovered in 0.7 and 2% from examined raw milk and ice-cream samples. Furthermore, *H. mustelae* was isolated in a percentage of 2% on HPSPA and 0.7% on Columbia agar from the examined ice-cream and raw milk samples, respectively.

On the other hand, *Helicobacter spp.* failed detection in the examined dairy farm raw milk, Damietta cheese and cooking butter samples.

Comparison between HPSPA and Columbia blood agar base for isolation of *Helicobacter spp.* revealed the superiority of HPSPA, however, the use of one medium was not enough for isolation of *Helicobacter spp.*

Plasmid analysis was performed on *H. pylori* isolates recovered from raw milk and ice-cream samples. All isolates (100%) carried plasmids of high molecular weight (over 2.6 kbp).

Furthermore, *H. pylori* isolates carrying plasmid DNA were analysed for possible relationship between the presence of plasmids and their antimicrobial sensitivity pattern and could detect the resistance of *H. pylori* isolates for 3 (37.5%) of antibiotic (metronidazole, nalidixic



acid and amoxicillin), and the sensitivity for 2 (25%) of antibiotic (tetracycline and cephalothin).

The effect of honey on survival of *H. pylori* was evaluated by adding different concentrations of honey (0.00, 10% and 20%) to the laboratory pasteurized and previously infected milk samples with *H. pylori* ( $7.7 \times 10^7$  cfu/ml). Each sample was divided into 2 portions, one was kept at room temp. (15°C) and the other was kept in refrigerator temp. (6°C). The samples were examined daily for *H. pylori* count until the samples were deteriorated.

The sensitivity of *H. pylori* to honey and temperature was demonstrated by a reduced count on HPSPA. The counts were decreased and reached  $2.6 \times 10^4$  and  $1.2 \times 10^3$  &  $1 \times 10^2$  and  $4 \times 10$  in 10% and 20% honey containing samples stored at 15°C and 6°C respectively.

The public health importance of the organism and the steps, which should be taken to control this organism in the dairy industry as well as the recommended sanitary measures, were also discussed.