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INCIDENCE OF SENSITIVITY OF HELICOBACTER PYLORI IN RAW AND POWDERED MILK TO SOME ANTIBIOTICS GROUPS USED IN EGYPT

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ABSTRACT

A total of 120 random samples of raw milk were collected from dairy farms, farmers houses, street vendors and milk powder (30 samples each) in clean, dry and sterile containers from different localities in Assiut city, Egypt. From January to October, 2018. The results showed that the Incidence of *Helicobacter* species in the examined raw milk collected from (dairy farms, farmers houses, street vendors) and milk powder samples were 4 (1.33 %) , 3 (1.00%) , 5 (1.66 %) and 1 (0.33 %) respectively , While the Incidence of *Helicobacter pylori* in the same samples of examined raw milk and milk powder samples were 2 (0.66 %) , 2 (0.66%) , 3 (1.00 %) and 0 (0.00 %) respectively , That means that the frequency distribution of *Helicobacter pylori* to *Helicobacter* species in the examined raw milk and milk powder samples were 50, 66.6, 60 and 0.00 % respectively , and the Degree of sensitivity of *Helicobacter pylori* to both Tetracycline 30mg , Cephalothine 30 mg and Ampicillin 10 µg were high sensitive (100 %) , while that the Degree of sensitivity of it to Clarithromycin 15 mg and Erythromycin 5 µg were moderate sensitive (75 %) and low sensitive to Augmentin 10 mg (50 %) . in other hand *Helicobacter pylori* were Resistant to both Amoxicillin 10 mg and Unasyn 20 mg .The healthy importance of *Helicobacter pylori* and methods of control are discussed.

Keywords: *Eschericia coli*, UHT Milk, Fermented Milk, IMVIC, TPC, yogurt

INTRODUCTION

Diarrhea can occur due to food and beverage poisoning, with the highest cause being the result of infection with various bacteria, viruses, or parasites. Bacteria that can cause this disease are Escherichia coli bacteria which are known as good bacteria in the digestive tract. Bacteria *Escherichia coli* is a species of bacteria with natural habitats in the digestive tract of humans and animals. But in reality in microbiology, not all types of Escherichia coli are good bacteria **(Delmas, 2015)**. Milk is a special food because of its delicacy and balanced composition. Milk contains substances needed by the body such as protein, fat, carbohydrates, minerals, and vitamins. UHT milk is one type of milk that has been processed. While fermented milk is milk with the addition of good bacteria needed by the body in the digestive system **(Delmas, 2011)**. The pollution that occurs in milk is caused by cows, unclean tools and unclean storage areas, dust, air, flies, and poor handling of personnel. After being released from cows, the content of microorganisms in milk is a function of age that determines the level of development of natural flora, the handling of milk that determines the types of organisms carried, and the storage temperature that determines the breeding speed of all types of organisms **(Dumalisile *et al.*, 2005).** High nutritional substances found in milk are good media for microbial growth. Microbial growth causes milk can not stand stored for long and is easily damaged. One way to preserve milk is fermentation treatment (Delmas, 2016). Fresh liquid milk which is widely used is UHT (Ultra High Temperature) milk. UHT milk is a milk product that is obtained by sterilizing milk at a minimum temperature of 135ºC for 2 seconds, without the addition of permitted food ingredients and aseptically packaged. This type of milk is usually packaged in a cup or glass with a variety of flavors. UHT milk is also packaged using cardboard boxes or shaped pads ***(Fischer et al., 2011)***. UHT milk and fermented milk are easily found by the public, both in small shops and large stores because these products are sold in the form of ready to drink packaging **(Widodo *et al.*, 2017)**. As a liquid beverage packaging product, UHT milk consumption tends to be more desirable than pasteurized milk. While fermented milk is also a product that is in demand because of its usefulness to the digestive system. So for the selection of pasteurized milk products tend to be less desirable because of constraints on the distribution channel **(Omara et al., 2018).** Pasteurized milk which requires the presence of a cold chain (cooling lane), tends to be durable and easily damaged. Bacterial pollution that occurs in liquid milk packaging that is after the packaging is opened. This can cause digestive disorders due to the influence of microorganisms that grow in the milk and some infection conditions such as urinary tract infections in children and infections of the digestive tract **(Dumalisile *et al.*, 2005).**

MATERIAL AND METHODS

Materials

The materials used in this study were UHT milk (samples A and B) and fermented milk (sample X) obtained from supermarkets in the Karawang area. Bacterial growth media are NA (Nutrient Agar), SIM (Sulfide-Indole-Motility), and MR-VP (Methyl Red-Voges Proskauer). The reagents used are Kovac reagents, methyl red reagents. Gram staining is the crystal violet, 96% alcohol, safranin, and aquadest.

Methods

Total Plate Count (TPC)

At Each Sample 1 mL is taken, then diluted using distilled water until dilution to 10^{-6}. Furthermore, From Each Sample 10^{-6} Dilution, 1 mL is taken to be inserted into a sterile petri dish, then poured nutrient agar (NA) liquid media and homogenized by sliding horizontal plates or forming the number eight and allowed to freeze. Incubation on milk 37ºC for 24 hours and all colonies that grow are counted as TPC.

Biochemical Test

From the dilution tube, make a scratch on the nutrient agar (na) media, and incubate at 37ºC for 18-24 hours. Observe the murky white colonies of NA.

Indol Test

Colonies from na media were inoculated in a sim-filled tube and incubated at 35ºC for 24 hours ± 2 hours. Then add a few drops of the kovac reagent until a red ring appears on the top layer for positive results and yellow rings for negative results.

Methyl Red (MR) Test

Colonies from na media were inoculated in tubes containing 10 ml mr-vp and inoculated at 35ºc for 48 hours ± 2 hours. Then add 2-5 drops of the mr indicator to the tube. Observe the presence of red for positive results and yellow for negative results.

Voges Prokauer Test (VP)

Pure bacterial isolate inoculated on mr-vp media and incubated for 24 hours at room temperature. Add 0.6 ml of 5% alpha naphtol solution followed by 0.2 mL of 40% KOH. Observe the color change for 30 minutes.

Citrate Test

Inoculation of pure bacterial isolate by zig-zag scratch using ose or using inoculation needle on simmons citrate media to tilt, then incubate for 24 hours at room temperature. Observe the color change from green to blue.

Gram Staining

In NA, take a murky white colony with ose. Then place it on the glass preparation, fix it on the fire bypassing the glass preparation on the fire twice. Drop gentian violet until the entire circle is covered, wait for 5 minutes. Clean over running water. Then drop the lugol and wait for 1 minute. Clean again above running water. Drop alcohol on the entire surface until no color wears off again. Clean again above running water. Drop safranin and wait for 2 minutes.
Clean again above running water. Dry preparations on a tissue.

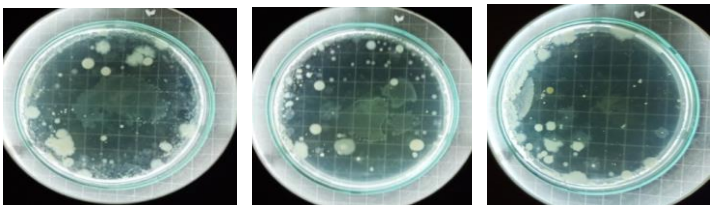
Microscopic examination

The first drop of immersion oil. Then check the preparations under the microscope of the smallest magnification first. After finding the colony lay, change the magnification up to 100 times. The appropriate form of escherichia coli is red, short trunk shape, and a single colony.

RESULTS AND DISCUSSION

Number of UHT Milk and Fermented Milk Colonies

Testing the number of colonies is done by using the cup calculation method with a 10-6 dilution. In the calculation of the number of colonies test results obtained are shown in Figure 1 with calculations using the cup calculation method.



Sample A
45 colonies

Sample B
60 colonies

Sample X
38 colonies

Figure 1 Calculation of the number of colonies in dilutions 10^{-6}

From the data above sample X which is a sample of fermented milk has the lowest number of colonies compared to samples A and B which are UHT milk samples. This can happen because the level of dilution is carried out low so that the growth of microorganisms becomes very numerous and difficult to do calculations, whereas for samples A and B the level of dilution is carried out high **(Caplan & Barbano, 2013)**. For samples A and B which are flavorful and non-flavored UHT milk, the results of the calculation of the number of colonies are in accordance with SNI provisions in 2009 concerning the maximum limit of microbial contamination, namely for flavored and non-flavored UHT milk <10 colonies / 0.1 mL or 100 colonies / 1 mL, while for sample X which is fermented milk is also in accordance with the provisions of SNI in 2009 concerning the maximum limit of microbial contamination that is max 10 / 0.1 mLor 100 colonies / 1 mL.

Identification of *Escherichia coli* bacteria

Tests in biochemical tests include various tests to determine the activity of microbial metabolism. Observation of metabolic activity is known to be the ability of microbes to use and decompose complex molecules such as starches, fats, proteins, and nucleic acids.

Table 1 Biochemical Test Results

Assays	Sample A	Sample B	Sample X
Indol	+	-	+
Methyl Red	+	-	+
Voges Prekauer	-	-	-
Citrat	-	-	-

Indol Test

The Indole test aims to identify the ability of bacteria to produce indole by using the enzyme tryptophanase. Indole production in the media is possible because of tryptophan. Tryptophan is an essential amino acid, which is oxidized by several bacteria which results in the formation of indole, pyruvic acid, and ammonia.

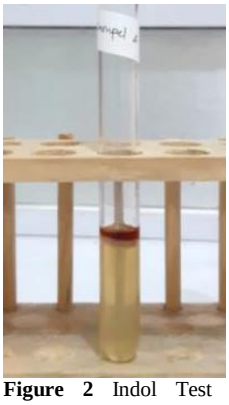


Figure 2 Indol Test sample A (+)

Figure 3 Indol Test sample B (-)

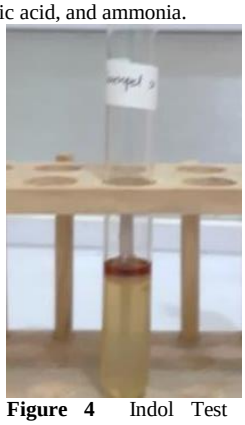


Figure 4 Indol Test sample X (+)

The Methyl Red (MR) Test

The Methyl Red (MR) Test aims to detect the ability of organisms to produce and maintain the final acid-stable product from glucose fermentation. Methyl red is a pH indicator, which remains red at pH 4.4 or less. The observations for the MR test yielded positive results as indicated by the presence of a red ring on the top.

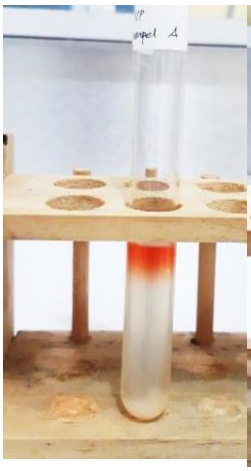


Figure 5 The Methyl Red Test sample A (+)

Figure 6 The Methyl Red Test sample B (-)

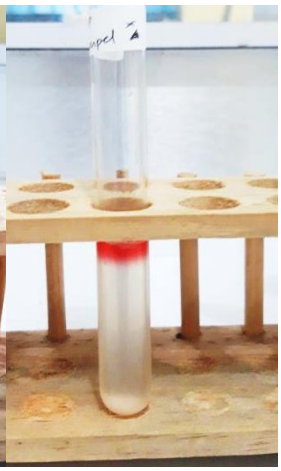


Figure 7 The Methyl Red Test sample X (+)

The Voges Proskauer (VP) test

The Voges Proskauer (VP) test is a test used to detect acetonin in a liquid culture of bacteria. This test is done by adding alpha-naphthol and potassium hydroxide. The red color indicates a positive result, while the yellow-brown or colorless color is a negative result. This test is negative for Escherichia coli because Escherichia coli ferments carbohydrates into acidic products and does not produce neutral products such as acetonin.

Figure 8 The Voges Proskauer test sample A, B dan X (-)

The citrate test

The citrate test aims to detect the ability of an organism to utilize it as the only source of carbon and energy. If the bacteria are able to use citrate as their carbon source, it will increase the pH and change the color of the culture medium from green to blue.

Figure 9 The citrate test sample A, B dan X (-)

Observation results for the citrate test yielded negative results indicated by the absence of color changes in the citrate test media. Biochemical tests are carried out to determine the physiological properties of bacteria. Based on biochemical tests the results showed that *Escherichia coli* bacteria were found in samples A and X. Positive tests on samples A and X were marked by the formation of a red ring on the upper layer of the indole test and the methyl red test. For negative results, sample B shows the results of non-*Escherichia coli* bacteria or other coliform bacteria, this negative result is also marked by the formation of yellow rings in the upper layer of the indole test and methyl red test. However, the results of this biochemical test have not shown specific results. Therefore the next test is gram staining and observation of the sample under a microscope. UHT milk samples that produce positive results indicate incompatibility with the provisions of the SNI in 2009 and SNI in 2014 that is contrary to the maximum limit of microbial contamination ***(*Martin *et al., 2011)***. This can occur because the process of making UHT milk in sample A does not meet the applicable requirements and the storage is not in accordance with the provisions or unfavorable research treatments such as samples contaminated with bacteria from outside during processing **(Boor *et al.*, 2017).** The test results on UHT milk samples indicate the presence of Escherichia coli bacteria is not in accordance with a study conducted by **(Cutrim *et al.*, 2017)** examining UHT milk against Escherichia coli bacteria which results in no microbial contamination in UHT milk. Whereas UHT milk in sample B produced negative results showing conformity with the conditions specified for the process of making UHT milk. These results indicate that the manufacturing process in sample B follows the applicable terms or conditions **(Coppa *et al.*, 2013)**. So the product produced is good because UHT milk is milk with a manufacturing process using heating techniques at high temperatures allowing the bacteria contained in the milk to die. For yogurt (sample X) that produces positive results containing Escherichia coli bacteria is a sample of fermented milk **(Aryana & Olson, 2017)**. This happens that the possibility of fresh milk used in the manufacture of fermented milk has been contaminated with Escherichia coli bacteria. So the product produced contains *Escherichia coli* bacteria **(Butler *et al.*, 2011)**. Because fermented milk is milk that is inoculated with lactic acid bacteria, the results of the methyl red test produce positive results because the lactic acid bacteria itself is able to ferment sugar into lactic acid **(Boor *et al.*, 2017)**. Another cause of the presence of Escherichia coli bacteria in the sample is the possibility of antibacterial activity against undesirable bacteria such as *Escherichia coli* **(Martin *et al.*, 2011)**. This can happen because the lactic acid production process runs fast so that the growth of other microbes that are not used in mixing can be inhibited like Escherichia coli. This statement is not necessarily true whether the bacteria contained in the sample used are Escherichia coli bacteria or other bacteria. For this reason, antibacterial activity testing must be done on the sample **(Koushki *et al.*, 2016)**.

Gram Staining and Microscope Test

Gram staining is used to identify bacteria. Bacteria stained by the gram method are divided into two groups, namely gram-positive bacteria and gram-negative bacteria. Gram-negative bacteria are bacteria that do not maintain purple dyes in gram staining. While gram-positive bacteria are bacteria that will maintain a dark purple color after rinsing with alcohol **(Tjatur *et al.*, 2015).**

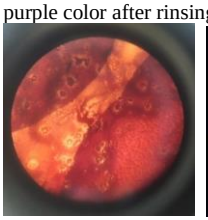


Figure 10 Preparations that are too thick (sample A)

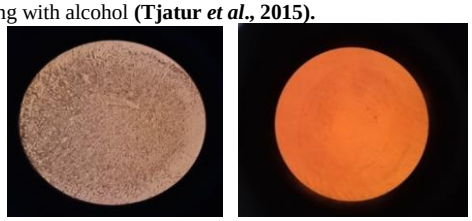
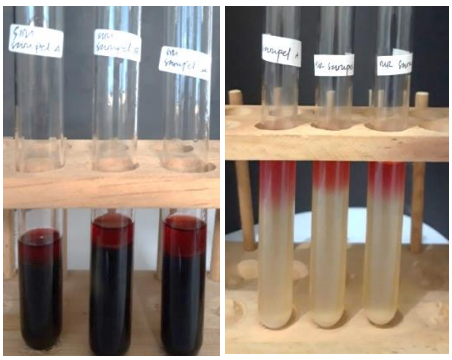


Figure 11 Preparations that are too thick (sample B and X)

The results of gram staining and testing under a microscope showed that the morphology did not appear to be due to the process of making preparations that were too thick (Figure 10), making preparations that were too thin (Figure 9), preparations that were not completely dry, bacteria that did not carry over from bacterial culture at the time of collection using an ose needle and improper treatment. It cannot provide information about the color of bacterial cells or show the nature of gram bacteria and cell shape. Errors in making preparations have also been explained by **(Widyastuti *et al.*, 2014)** that things that often fail microscopy testing is excessive culture taking, preparations that are too thick, preparations that are too thin, preparations that have not been completely dry resulting in microorganisms which were observed to be poorly formed, as well as a less aseptic treatment aimed at avoiding contamination. In these results did not show specific results, then conducted a comparison test with samples inoculated with *Escherichia coli* bacteria **(Butler *et al.*, 2011)**.



Figfure 12.a

Figure 12.b

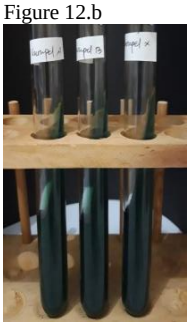


Figure 12.c

Figure 12. d

Figure 12 Observation of comparative test results (a) indole (+), (b) methyl-red (+), (c) voges prokauer (-), (d) citrate (-)

In the picture above can be seen the results of the four tests obtained two tests showed positive results and two tests showed negative results. This is by the applicable provisions that the test that shows the presence of *Escherichia coli* bacteria is by obtaining a positive result in the indole test and methyl red test, and the next two tests show negative results.

CONCLUSION

1. Based on the research that has been done, it can be concluded that the sample containing *Escherichia coli* bacteria is contained in sample a uht milk and sample X which is yogurt / fermented milk.
2. The results of the calculation of the colony by the SNI of 2009 concerning the maximum limits of microbial contamination in food namely 10 colonies / 0.1 mL or 100 colonies /1 mL.

REFERENCES

Aryana, K. J., & Olson, D. W. (2017). A 100-Year Review: Yogurt and other cultured dairy products. *Journal of Dairy Science*, *100*(12), 9987–10013. https://doi.org/10.3168/jds.2017-12981

Boor, K. J., Wiedmann, M., Murphy, S., & Alcaine, S. (2017). A 100-Year Review: Microbiology and safety of milk handling. *Journal of Dairy Science*, *100*(12), 9933–9951. https://doi.org/10.3168/jds.2017-12969

Butler, G., Nielsen, J. H., Larsen, M. K., Rehberger, B., Stergiadis, S., Canever, A., & Leifert, C. (2011). The effects of dairy management and processing on quality characteristics of milk and dairy products. *NJAS - Wageningen Journal of Life Sciences*, *58*(3–4), 97–102. https://doi.org/10.1016/j.njas.2011.04.002

Caplan, Z., & Barbano, D. M. (2013). Shelf life of pasteurized microfiltered milk containing 2% fat1. *Journal of Dairy Science*, *96*(12), 8035–8046. https://doi.org/10.3168/jds.2013-6657

Coppa, M., Ferlay, A., Chassaing, C., Agabriel, C., Glasser, F., Chilliard, Y., Borreani, G., Barcarolo, R., Baars, T., Kusche, D., Harstad, O. M., Verbič, J., Golecký, J., & Martin, B. (2013). Prediction of bulk milk fatty acid composition based on farming practices collected through on-farm surveys. *Journal of Dairy Science*, *96*(7), 4197–4211. https://doi.org/10.3168/jds.2012-6379

Cutrim, C. S., Barros, R. F. de, Franco, R. M., & Cortez, M. A. S. (2017). Escherichia coli O157:H7 SURVIVAL IN TRADITIONAL AND LOW LACTOSE YOGURT DURING FERMENTATION AND COOLING PERIODS. *Ciência Animal Brasileira*, *18*(0), 1–9. https://doi.org/10.1590/1089-6891v18e-39554

Delmas. (2011). Contaminants of Milk and Dairy Products: Contamination Resulting from Farm and Dairy Practices. *Encyclopedia of Dairy Sciences: Second Edition*, *January 2018*, 887–897. https://doi.org/10.1016/B978-0-12-374407-4.00104-7

Delmas. (2015). Escherichia coli: The Good, the Bad and the Ugly. *Clinical Microbiology: Open Access*, *04*(02), 2–4. https://doi.org/10.4172/2327-5073.1000195

Delmas. (2016). Microbiological quality of pasteurized milk on expiration date in Tehran, Iran. *Journal of Dairy Science*, *99*(3), 1796–1801. https://doi.org/10.3168/jds.2015-10400

Dumalisile, P., Witthuhn, R. C., & Britz, T. J. (2005). Impact of different pasteurization temperatures on the survival of microbial contaminants isolated from pasteurized milk. *International Journal of Dairy Technology*, *58*(2), 74–82. https://doi.org/10.1111/j.1471-0307.2005.00189.x

Fischer, W. J., Schilter, B., Tritscher, A. M., & Stadler, R. H. (2011). Contaminants of Milk and Dairy Products: Contamination Resulting from Farm and Dairy Practices. *Encyclopedia of Dairy Sciences: Second Edition*, *January 2018*, 887–897. https://doi.org/10.1016/B978-0-12-374407-4.00104-7

Koushki, M., Koohy-Kamaly, P., Azizkhani, M., & Hadinia, N. (2016). Microbiological quality of pasteurized milk on expiration date in Tehran, Iran. *Journal of Dairy Science*, *99*(3), 1796–1801. https://doi.org/10.3168/jds.2015-10400

Martin, N. H., Ranieri, M. L., Murphy, S. C., Ralyea, R. D., Wiedmann, M., & Boor, K. J. (2011). Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk. *Journal of Dairy Science*, *94*(3), 1211–1222. https://doi.org/10.3168/jds.2010-3915

Omara, T., Ndyamuhaki, S., Kigenyi, E., Kagoya, S., & Biira, F. (2018). Comparative Evaluation of the Microbial Safety of Boiled Locally Vended Ready-to-Drink, HTST Pasteurized and UHT Bovine Milk Sold in Nakawa Division of Metropolitan Kampala. *International Journal of Food Sciences and Nutrition*, *3*(6), 185–189. https://doi.org/10.20944/preprints201811.0445.v1

Tjatur, A., Krisnaningsih, N., & Yulianti, D. L. (2015). *Improving The Quality of The Yoghurt With The Addition of Honey*. *November*, 205–211.

Widodo, Handaka, R., Wahyuni, E., & Taufiq, T. T. (2017). The quality of fermented milk produced using intestinal-origin lactic acid bacteria as starters. *International Food Research Journal*, *24*(6), 2371–2376.

Widyastuti, Y., R., & Febrisiantosa, A. (2014). The Role of Lactic Acid Bacteria in Milk Fermentation. *Food and Nutrition Sciences*, *05*(04), 435–442. https://doi.org/10.4236/fns.2014.54051