



Investigation of Antibiotic Residues In Pork And Chicken Advertised As Hygienic Sold In Supermarket In Bangkok, Thailand

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Abstract

Background: Antibiotics resistance is a part of food safety. 700,000 people worldwide die from antibiotics resistance each year. In a 2012 study, 130 samples, including 70 chicken and 60 beef samples which are both raw and ready to eat, were tested for antibiotic residue. The results found that 51 samples out of 130 samples tested positive in antibiotic residue which totaled to 39%. Therefore, the researchers want to investigate that the products they claim to be hygienic have no chemical and antibiotic residues.

Objective: To detect antibiotic residues in pork and chicken that were advertised as hygienic products. The researchers want to investigate that the products they claim to be hygienic have no chemical and antibiotic residues.

Methods: The samples were tested by antibiotic residue detection kits from the Department of Medical Sciences, Thailand. These detection kits have 93% accuracy, 78.9% sensitivity, and 96.7% specificity. 72 samples of raw pork and chicken, which were advertised as organic, were tested for Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide and Penicillins.

Results: In the 72 samples of raw pork and chicken, we found no antibiotic groups in any of the samples.

Conclusion: The samples of raw pork and raw chicken which advertised as hygienic products were found not containing any antibiotic residues.

Keywords: drug residues, food safety, antibiotics

Introduction

700,000 people die per year from diseases related to antibiotic resistance all over the world. If this trend continues until 2050, a total of 10 million people will be affected. In Thailand alone, it has been estimated that there will be approximately 880,000 health cases related to antibiotic resistance and 38,000 deaths every year (1).

Antibiotic resistance is caused by bacteria adapting to become immune against antibiotics. Causes of antibiotics resistance are the inappropriate use of antibiotics in the treatment or antibiotic residues in food products such as meat and vegetables. Whether it's for prevention of infectious diseases in humans or to increase the effectiveness of agriculture, improper use in terms of incorrect amounts or ways can all increase the presence of antibiotic resistance (2).

We can help resolve drug resistance problems by 1) stopping a creation antibiotic resistant bacteria 2) stopping a spread of antibiotic resistance and 3) stopping receiving antibiotic bacteria. For stopping a creation of antibiotic resistance, it is to use antibiotics appropriately at doctor's prescription. Additionally, people have to consider food safety and prepare food hygienically by following the WHO i.e. the five keys to food safety including to keep clean, to separate raw and cooked food, to cook thoroughly, to keep food at safe temperature with a use of clean water and safe raw materials, and to choose foods that have been produced without the use of antibiotics for growth promotion or disease prevention in healthy animals. To stop the spread of antibiotic resistance, the medical personnel have to wash hands every time when touching patients and use the principle of sterile technique and aseptic technique to separate patients; however, the medical equipment must be clean and sterilised (3)(4).

Food safety comes into play with antibiotic resistance. Humans need to consume food. Food is important for human life. The human body needs food for energy, warmth, growth and repair. We need nutrients on a daily basis in order to stay healthy, thus, the first concern regarding antibiotic resistant bacteria is food safety. Safe food keeps humans healthy. Food safety refers to handling, preparing and storing food in a way to best reduce the risk of individuals becoming sick from foodborne illnesses.

Antibiotic and chemical residues can be found in food globally. In a 2012 study, 130 samples, including 70 chicken and 60 beef samples, raw or cooked, were tested for antibiotic residue. The samples were collected from supermarkets in Bangkok and the nearby areas. Screening kits were used to screen Tetracycline, Penicillin and Sulfonamide groups of antibiotics. The results found that 51 samples out of 130 samples tested positive in antibiotic residue which totaled to 39%. Raw chicken tested positive in 50% of the samples(5). In another experiment, the 50 pork samples were collected from traditional markets in Denpasar and Bali, Indonesia. Four of the samples were positive for antibiotic residues; two of the samples were positive in trachylinic and other two contained penicilline. Overall, 8% of the samples had positive results showing antibiotics(6). Furthermore, there was

another study in Turkey which used 127 samples of chicken breast meat from local markets in Ankara, Turkey. The samples were collected from July 2010 to September 2010. The conclusion was that, out of 58 samples, approximately 46% of chicken breast meat had the residues of quinolone antibiotic (7).

In addition, vegetables and fruits from local farms have been found to contain chemical residues. In Thailand, 64% of the products from shopping malls and supermarkets in Bangkok and four other provinces were found to be contaminated with pesticides (8). Another study was conducted in Saudi Arabia in March to September of 2018 with 211 vegetable samples from supermarkets from in the Asir region. 80 different pesticides were being studied using the process of ultrahigh-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) and gas chromatography–tandem mass spectrometry (GC-MS/MS) and was extracted using multi-residue method which was the QuEChERS method. Results yielded 145 samples out of all the samples found pesticide residue which totaled to 68.7%(9). Another study used vegetable samples collected from local markets from 11 different districts in Bangladesh. Pyrethroid and organophosphate pesticides were being monitored in an experiment. Out of the 135 samples, 37 of them were found to have pesticide residue. 33 of the samples which had pesticide residue have higher levels of pesticide than the level of MRLs (Maximum Residue Limits) (10).

Regardless, there are several brands and sellers that are selling hygienic pork and chicken around Bangkok.

We researchers aimed to investigate if the advertised products have no chemical and antibiotic residues.

Objectives

To detect Antibiotic residues in pork and chicken that are advertised as hygienic products.

Study Method

Instrument and Tools

Sampling

This research focuses on detecting antibiotic residue in 72 samples by gathering a group of pork and

chicken samples by convenience sampling method to test and find antibiotics that are residual inside the samples. 36 samples of fresh pork and 36 samples of fresh chicken samples which were advertised as hygiene products were bought from different super markets from different locations in Bangkok. In the process of finding residual antibiotics, researchers operated by using antibiotic residue detection kits from the Department of Medical Sciences, which were produced by Rodejanarug Pharmaceutical. These detection kits have 93% accuracy, 78.9% sensitivity, and 96.7% specificity. In addition, these detection kits can analyse the least amount of antibiotics in meats that are globally acceptable. The type of antibiotics that can be examined by these kits are Penicillins, Amoxicillin, Tetracyclines, Oxytetracycline, Chlortetracycline, Gentamicin, Neomycin, Strepto-mycin, Sulfadimethoxine, Thyroxin, Erythromycin and Bacitracin (11).

Test Procedure

Test Procedure

1. Take a sample of minced meat (Chicken or Pork) and place 5g into a 30ml centrifuge

- 10.1 Test for drug residues in the **tetracycline group** (Adjusting the pH to 6.5)

- Chicken:

- Drop 1 drop of N NaOH (33 μ l) in the clear part (that has a pH of ~6.1 and a volume of 3.75 ml)

- Pork

- Drop 1 drop of N NaOH (33 μ l) in the clear part (that has a pH of ~5.8 and a volume of 2 ml)

- If the starter pH was 5.5, drop 1 drop of N NaOH (33 μ l) in the clear part (that has a volume of 1.5 ml)

- 10.2 Test for drug residues in the **penicillin group** (Adjusting the pH to 6.5)

- Chicken:

- Drop 1 drop of N NaOH (33 μ l) in the clear part (that has a pH of ~6.1 and a volume of 5ml)

- Pork

- Drop 1 drop of N NaOH (33 μ l) in the clear part (that has a pH of ~5.8 and a volume of 2 ml)

- 10.3 Test for drug residues in the **macrolide, aminoglycoside and sulfonamide group** (Adjusting the pH to 7.0)

tube. Repeat this twice so you have three centrifuge tubes with the same meat samples.

2. Add 5 ml of Extract A (tetracycline group) to one of the centrifuge tubes
3. Add 5 ml of Extract B (macrolide, aminoglycoside and sulfonamide group) to another centrifuge tube
4. Add 5 ml of Extract C (penicillin group) to the final centrifuge tube
5. Shake every tube vigorously by hand or a shaker for 10 minutes\
6. Heat the tubes in a water bath (with a temperature with 60°C) for 5 minutes.
7. Quickly remove them from the water bath and cool them after the time is up
8. Centrifuge at 3,000 - 4,000 cycles for 15 minutes
9. Obtain the clear liquid of the sample
10. Adjust the pH of the transparent liquid for testing:

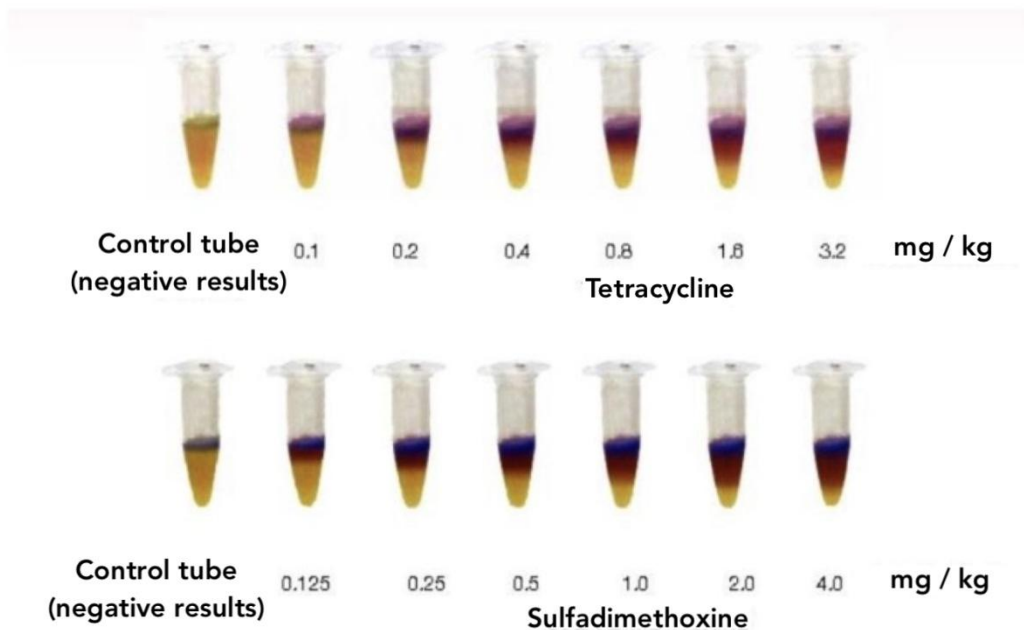
- Chicken:
 - Drop 1 drop of N NaOH (33µl) in the clear part (that has a pH of ~6.5 and a volume of 5ml)
- Pork
 - Drop 1 drop of N NaOH (33µl) in the clear part (that has a pH of ~6.1 and a volume of 1.5ml)

11. Adjust the pH of a control sample following the steps of 10.1 - 10.3 to compare with the results.

(In the case that the pH of meat samples has already reached the given range, there is no need to adjust the sample.)

How to Interpret the result.

How to interpret results



Result

A total of 72 samples which consisted of 36 pork samples and 36 chicken samples were tested for antibiotic residues; Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide and Penicillins. No sample was detected of antibiotic residues.

Table 1. Number of sample detected drug residues Tetracyclines, Macrolide, aminoglycoside, Sulfonamide and Penicillins (N=72)

No.	Type of Meat	Total sample	Number of Sample detected drug residues

			Tetracyclines	Macrolide, Aminoglycoside, Sulfonamide	Penicillins
1	Fresh Pork	36	0	0	0
2	Fresh Chicken	36	0	0	0
	Total	72	0	0	0

Discussion

From our study of the detection of antibiotics: Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide and Penicillins in 72 samples of fresh pork and fresh chicken meat, which were advertised as chemical and hormone free, we have found that there were no group of any antibiotics in all 72 samples. This may be due to the farms that sell fresh pork and fresh chicken either did not use antibiotics in their farming or had antibiotics discontinued in the last two weeks, making the group of antibiotics undetectable. Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide, and Penicillins

However, in other studies, antibiotics were detected in raw pork, chicken and beef samples in several areas during 2017-2021. A study was conducted by Jesada Jiwakanon *et al.* focusing on antibiotic residues in pig meat in northeastern Thailand. From their 469 samples during 2017-2018, antibiotics were found in 16.6% of pork samples. The antibiotics found were mainly tetracycline and penicillin (12). After this, chicken meat and chicken livers were randomly examined for residual leftovers of 3 antibiotics from 3 groups, namely group 1, Fluoroquinolone group: Enrofloxacin, group 2, Tetracycline group: Doxycycline, Group 3 Beta - Lactam: Amoxicillin. This was tested from 9th of June to 15th of June in 2018 by the Foundation for Consumers. Out of 62 samples, 26 samples contained antibiotic residue, or more than 40% (13). Sathitkhun Maitrijit *et al.* studied antibiotic infection in fresh pork and cattle sold at the Muang District Fresh Market, Phitsanulok Province, from July 2019 to October 2020. There were 37 samples of fresh pork and 14 samples of cattle. Antibiotic residues were found in 1 sample of pork, accounting for 2.7% and 1

sample of cattle, accounting for 7.1% (14). From a summary of the results of the Food Safety Integration Project for the fiscal year 2019, it was found that 78.9% of fresh chicken meat samples 65.6% of the pork samples and the final beef samples showed antimicrobial resistance (15). This can have several reasons as to why: 1) infection prevention. Because Thailand is a country with high humidity, there is a high rate of disease infection in farms by default. 2) Antibiotics were used incorrectly and mixed with the food for animals. 3) Inappropriate use of the drugs. 4) No stopping the drug for a specified period of time causing drug residues in animal tissues in dangerous amounts. Finally, 5) there is no residual antibiotic test before it reaches the shelves for consumers (16).

Antibiotics used in animals are the same class as those used in humans, such as Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide Penicillins, and Fluoroquinolones. Drug-resistant bacteria that occur in animals can be passed on to humans in three main ways: 1) consuming meat or animal products, 2) contact with animals, especially herdsman, and 3) exposure from animal environments, such as in water sources and soil, etc. Therefore, frequent consumption of meat with antibiotic residues may result in drug-resistant bacteria (16).

From the results of our study, it was concluded that in order to reduce the problem of finding antibiotic residues in meat, we must place emphasis on the correct use of antibiotics. We must strictly follow the directions when we are using these medications for ourselves, use the correct drug for the right disease, right size, right time, and choose to eat hygienic meat. However, in the animal farm industry, this would be immensely difficult to avoid, due to climatic factors which makes it easy to get infected,

and the cost of raising animals can be much higher if no antibiotics are used in their farms.

Limitation

The sample size in this study was too small, and due to the limited number of organic pork and organic chicken producers in Thailand, the samples may not be very diverse. Sampling was performed over a 1-month period, so results may not be conclusive in general for organic pork & chicken. Another aspect is that this test was performed using a test kit, which means that results may differ from modern laboratory testing.

Conclusion

In our study of the detection of antibiotic groups: Tetracyclines, Macrolide, Aminoglycoside, Sulfonamide and Penicillins, in 72 samples of raw pork and chicken which were advertised as organic, we found no antibiotic groups in any of the 72 samples.

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References

1. AMR Thailand. Antibiotic Resistance Situation in Thailand. Available from: <https://amrthailand.net/Home/Thailand> [Last accessed on 2022 Mar 1]
2. Tongrod W. Antimicrobial Resistance (AMR). Pharmaceutical Association of Thailand under Royal Patronage. 2018; Available from: <https://ccpe.pharmacycouncil.org/showfile.php?file=46> [Last accessed on 2022 Mar 1]
3. PAHO. PAHO/WHO recommends five keys to safer food for a healthy holiday season. Available from: <https://www.paho.org/en/news/21-12-2015-pahowho-recommends-five-keys-safer-food-healthy-holiday-season> [Last accessed on 2022 Mar 1]
4. Khoka A. Antibiotics and antibiotic resistance. *Journal of Medicine and Health Sciences*.2020;27(2). Available from: <https://he01.tci-thaijo.org/index.php/jmhs/article/download/244782/166171/864571> [Last accessed on 2022 Apr 6]
5. Bilatu Agza Gebre. Qualitative screening of antibiotic residues and identification of antibiotic resistant salmonella from raw and ready to eat meat in Thailand. *International Journal of Advanced Life Sciences: Volume 5; Issue 1; 2021 Nov. Pages 51-64.* Available from: https://www.researchgate.net/publication/236847444_Qualitative_screening_of_antibiotic_residues_and_identification_of_antibiotic_resistant_salmonella_from_raw_and_ready_to_eat_meat_in_Thailand
6. Siswanto S, Sulabda IN. Residue of Tetracycline and Penicillin Antibiotic On Pork In Denpasar Bali. *JVeterinary and Animal Sciences*. 2019 Jun 3;2(2):79. Available from: [<https://ojs.unud.ac.id/index.php/JVAS/article/download/59228/34360/> accessed 25 April 2022]
7. Buket Er, Fatma Kaynak Onurdağ, Burak Demirhan and et al. Screening of quinolone antibiotic residues in chicken meat and beef sold in the markets of Ankara, Turkey. *Poultry Science: Volume 92, Issue 8, 1 August 2013, Pages 2212-2215.* Available from <https://reader.elsevier.com/reader/sd/pii/S0032579119388261?token=C448F2D1505EE09EB56D84D9B762B95891FA55B42861AC6EEFB034DE8D9BEE035D759A5F6E521A79AC6797D4F00329BF&originRegion=eu-west-1&originCreation=20220507075521>
8. Penchan Charoensuthipan. 64% of veggies “unsafe” due to pesticides. *Bangkok Post* [Internet]. [cited 2022 May 1]; Available from: <https://www.bangkokpost.com/thailand/general/1366435/64-of-veggies-unsafe-due-to-pesticides>
9. Ramadan MFA, Abdel-Hamid MMA, Altorgoman MMF, AlGaramah HA, Alawi MA, Shati AA, Shweeta HA, Awwad NS. Evaluation of Pesticide Residues in Vegetables from the Asir Region, Saudi Arabia. *Molecules*. 2020 Jan 3;25(1):205. doi: 10.3390/molecules25010205. PMID: 31947847; PMCID: PMC6982748. Available

- from:
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6982748/>
10. Rahman M, Hoque MS, Bhowmik S, Ferdousi S, Kabiraz MP, van Brakel ML. Monitoring of pesticide residues from fish feed, fish and vegetables in Bangladesh by GC-MS using the QuEChERS method. *Heliyon*. 2021 Mar 8;7(3):e06390. doi: 10.1016/j.heliyon.2021.e06390. PMID: 33869825; PMCID: PMC8035496.. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8035496/>
 11. Asian Medic. Determination of Drug Residue in Meat Test Kit; 2022. Available from: <https://asianmedic.com/wp-content/uploads/2017/07/ยาปฏิชีวนะและสารต้านจุลชีพตกค้างในเนื้อสัตว์.pdf>. [Last Accessed 2022 Mar 1]
 12. Jatesada Jiwakanon, Seri Kang-air, Sarthorn Porntrakulpipat, and et al. Antibiotic residues in pork in the northeast of Thailand. *KKU Veterinary Journal*: Volume 31; Issue 1; 2021. Available from: <https://he01.tci-thaijo.org/index.php/kkuvetj/article/view/250392/170953>
 13. Thai PBS. Found Antibiotic Residues in Chicken meat from random detection; 2019. Available from: <https://news.thaipbs.or.th/content/273475>. [Last Accessed 2022 Feb 25]
 14. Satitkoon Maitreejit, Sittinee Patomkamtorn, Songsak Srisanga and et al. The antibiotic residues in raw pork and beef sold at the fresh marketsin Muang District, Phitsanulok Province. *JPHNU*. Vol.3 No.3 September-December 2021. Available from: <https://he01.tci-thaijo.org/index.php/JPHNU/article/view/250266/171246>
 15. Department of Medical Science. Food Safety Report: Budget Year 2019. Available from: <http://bqsf.dmsc.moph.go.th/bqsfWeb/wp-content/uploads/2017/Publish/e-book/รายงานบูรณาการอาหารปลอดภัย%202562/Food62Eb/foodSafty62.html#p=72>. [Last Accessed 2022 Mar 1]
 16. Jaiporn Poomkam. (Un)safe food, the consequences of using antibiotics in livestock. *FDA Journal* : September-December 2012,8-11. Available from: <https://www.tci-thaijo.org/index.php/fdajournal/article/download/138475/102928>