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Determination Of Drug Residue In Fresh Pork And Chicken Sold In Bangkok, Thailand

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Abstract

Background: .The agricultural industry uses antibiotics in their products in order to keep their product resistant to the bacteria. Somehow, they overuse it and it causes antibiotic residue in our food which makes us less resistant to developed bacteria. Our purpose in this lab is to find out and observe bacteria in meat products.

Objective: To detect antibiotic residues in pork and chicken

Study Methods: Samples of fresh pork and fresh chicken were sampled at a fresh market in Bangkok using an antibiotic test kit of the Department of Medical Sciences.

Results: 23 pork and 9 chicken samples from fresh markets were tested to find antibiotic residues; Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin. The result showed that 14 (60.87%) pork samples were detected with Tetracycline in it where 6 sample exceeded MRLs. 17 (73.91%) pork samples were detected with Macrolide, Aminoglycoside, Sulfonamide where 7 samples exceed MRLs., And 17 (73.91%) were found to contain Penicillin in it where 6 pork samples exceeded MRLs. 6 (66.67%) chicken samples were found to have Tetracycline in it which 3 (50%) exceed MRLs. Macrolide, Aminoglycoside, Sulfonamide had been found in 8 (88.89%) chicken samples which 3 (37.5%) exceeded MRLs. And 4 chicken samples were detected with Penicillin which 1 sample exceeded MRLs.

Conclusion: Most of the pork and chicken samples were detected antibiotic residue in it. Most of it exceed the maximum residue limit (MRLs).

Keywords: antibiotic residues, fresh pork, fresh chicken

Introduction

Antibiotics have long been used in the agriculture industry as an accepted practice to help treat and promote the growth of livestock. The agricultural industry is the largest consumer of antibiotics, responsible for around 70% of all antibiotic consumption. This large consumption of antibiotics includes those that are justified and those that are considered to be overuses, which can result in antibiotic residues ending up in meat products that can later be the cause for antibiotic resistance in humans and contaminated meats. The consumption of meats contaminated with high levels of antibiotic residue creates a health risk for the consumer. Antibiotic resistance is the ability for bacteria to be able to defeat the drugs that are used against them and resistance develops through the exposure of drugs to the bacteria. Overuse increases the amount of residue left in meat, increasing bacterial exposure to antibiotics, further increasing the chances of developing antibiotic resistance to the drug[(1)].

There have been reports of leftover residues found in supermarkets and local shops which vary from country to country. Thailand has had regular yearly testing of antibiotic residues in the past years. One study conducted in 200 from July to December by

Anong Bintwihok and colleagues testing poultry, pork and cow's milk for antibiotic residue levels. The study tested 200 samples of each type combining for a total of 600 samples sourced from 10 separate places. The study tested for levels of tetracycline, gentamicin, dihydrostreptomycin, chlortetracycline and oxytetracycline through ELISA and HPLC. The results have found that the concentrations found in the majority of samples are lower than the maximum residue limit (MRL) set up by the Codex ALimentarius Commission (CAC)[(2)].

The Ministry of Public Health announced the maximum amount of antibiotic residues in pork and chicken (MRL) at 200 micrograms per kilogram in 2007[(3)]. A study conducted on February 15, 2017 by The Independent Committee for Consumer Protection (ICCP) Indy Consumer Magazine, Foundation for Consumer, and Thai Drug Watch Organisation[(4)] tested 15 samples of pork collected from 6 fresh markets in bangkok, 8 malls and 1 online shop using an in-house method SOP LBFD-10556. The study found a total of 2 samples containing Chlortetracycline residue at 20.28 ug/kg and 42.57 up/kg. All samples were under the MRL. Another finding by the Secretary of Foundation for Consumer[(5)] found 18 samples of fast food contaminated with doxycycline at 13.73 ug/kg. While the concentration is under the limit of 200 ug/kg, the residual antibiotic can still cause allergic reactions and antibiotic resistance causing harm to the consumer. Fast food companies are being called on by consumer organisations to reduce and stop antibiotic use in the animal husbandry process to minimise health risks. One survey in 2019 by the Department of Medical Sciences[(6)] researched antibiotic resistant bacteria in 3 types of meat, pork, beef and poultry and found a total of 6 bacterias of interest: E. coli, E. faecalis, E. faecium, S. aureus, Salmonella spp. and Campylobacter spp. The survey found antibiotic resistant bacteria in 91.2% of poultry, 81.5 percent of beef and 77% of pork. The survey contained meats from 3 sources, fresh markets, malls, and local brands. 98.5% of meats from the fresh markets were contaminated with resistant bacteria while 86.7% of meats from malls were contaminated and only 56.5 percent of meats from local brands were contaminated. In total, 78.9% of poultry, 65.6% of pork and 51.8 percent of beef were contaminated. This study combined with the others show how the use of antibiotics have increased to an alarming level, dangerous to consumers and their health.

Foods containing high levels and concentrations of antibiotic residue can be harmful to the consumer's health and exacerbate the growing problem of antibiotic resistance in bacteria. Bacteria developing resistance to these antibiotics is a large problem because of the fact that we use the same antibiotics on livestock that we use on humans including but not limited to Tetracyclines, Macrolides, Penicillins, Sulfonamides and Fluoroquinolones. Antibiotic resistant bacteria that develop in animals can easily be transferred to humans through consumption, close contact, and contamination of the environment. Infections involving these bacteria can be much worse than their unresistance counterparts causing increased damage, complications and cost in treating the infection[(7)].

Objective:

1. To detect antibiotic residues in Chicken and pork

Instrument and tools

Sampling

This study focused on detecting antibiotic residue in fresh pork and chicken sold in supermarkets in the Bangkok area. Random sampling method was used to buy fresh pork from different districts in Bangkok. A total of 32 samples which comprised 23 pork samples and 9 chicken samples were bought from 8 different districts in Bangkok. Procedure testing residual antibiotics, researchers conducted the test 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, Streptomycin, Sulfadimethoxine, Thyroxin, Erythromycin and Bacitracin[(8)].

Procedure

Tools

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1) Test tubes (50)
2) Pipette (10)
3) Laboratory Water Bath
4) Incubator (Culture)
5) Extraction Liquid (Depending on the Type of Drug)

Stocking and preparing meat samples

1) Storing 5 gram of sampling meats or offals into a 30 milliliter centrifuge tube or any centrifuge tube with a size of more than 30 millilitre, depending on type of antibiotic residues.
2) Adding 5 milliliters of extract solutions to each of the samples. Add the extract solution A (tetracycline group) for shrimps and fish, and extract solution B (macrolide group, aminoglycoside ,and sulfonamide group) and extract solution C (penicillin group) are for testing chicken, pork, beef, and offals.
3) Shake the tube by hand or with a shaker for 10 minutes, before heating it in a water bath at 60 degree Celsius for 5 minutes and let it cool down afterwards.
4) Centrifuge at 3000 to 4000 rounds for 15 minutes for separation ,leaving the sample with the transparent part.
5) Before bringing it for testing, adjust the transparent part to a suitable pH first.
6) Compare the samples by adjusting the pH and compare it with a control sample that has a negative result.
7) If the sample has a pH of 6.5 for tetracycline, 6.5 for penicillin, and 7.0 for macrolide, aminoglycoside, and sulfonamide, then pH adjustments are not needed.

Procedure for testing meat sample

1) Using a dropper, drop 4 drops of the meat samples which were extracted (excluding air bubbles) into the testing set. Moreover, testing sets with trimetoprim are for finding sulfonamide.
2) Drop 4 drops of the negative control sample into one test tube
3) Ripe the testing set from step 1 and 2 in the water bath / incubator at the temperature of 64 ± 2℃. Fish and shrimps should be left for 2 hours and 30 minutes or more. Chicken, pork, and beef should be left for 2 hours and 45 minutes or more. The results could also be read on the test tube by keeping the culture medium in the tube stay under the water level until the negative control sample changes color from purple to yellow.

How to read and interpret results

1) Test tubes with a result of pure yellow means there are no residues.
2) Test tubes with slightly purple color or separates the color yellow and purple clearly means residue is present.

Efficiency of the testing set

This testing set has an accuracy of 93%, an sensitivity of 78.9%, a specificity rate of 96.7%, and has the ability to detect at least 12 different types of drug residues which detects the level of the drug residues that are acceptable according to the international standard. Penicillin, amoxicillin, tetracycline, oxytetracycline, chlortetracycline, gentamicin, neomycin, strep, tomycin, sulfadimethoxine, tyrosine, erythromycin, and bacitracin are drugs that may be detected.

Shelf life of test sample

Store all the equipment which is used for testing at a refrigerated temperature of approximately 4-8˚C for three months, except for the sets that are used for testing sulfonamide antimicrobials. It should be stored for not more than 20 days.

After experiment

Pour disinfectant solutions into the test tube until it covers the culture medium before leaving it for 30 minutes, or boil the test tubes with the lids covered for 15 minutes. Autoclaves can also be used and throw away the tubes afterwards.

Result

Table 1 showed the result of detecting three groups of antibiotic residues, which are Tetracycline, Macrolide, Aminoglycoside, Sulfonamide, and Penicillin, in pork from fresh markets in Bangkok in a number of 23 samples. It was found that 14 samples

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of the total (60.87%) detected Tetracycline and 6 of them contain more Tetracycline than MRLs which are 43% of the samples that were tested positive. 17 samples of the total (73.91%) detected Macrolide, Aminoglycoside, and Sulfonamide and 6 of them contain more Macrolide, Aminoglycoside, and Sulfonamide than MRLs which are 35% of the sample that were tested positive. 17 samples of the total (73.91%) detected Penicillin and 7 of them contain more Penicillin than MRLs which are 41% of the samples that were tested positive.

Table 1 Number of Pork samples detected drug residues/ percentage of sample detected drug residues (A, B, C) categorised by the source of the samples (N=23)

Location	Total Pork Sample	Tetracycline			Macrolide, Aminoglycoside, Sulfonamide			Penicillin		
		Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs
Prawet	2	2	1	50%	2	1	50%	1	0	0%
Bangkapi	2	2	1	50%	2	1	50%	0	0	0%
Dindaeng	1	1	1	100%	1	0	0%	1	1	100%
Wangthonglang	1	1	1	100%	1	0	0%	1	1	100%
Thanyaburi	3	3	0	0%	3	0	0%	3	0	0%
Promprabsatupai	2	2	1	50%	1	1	100%	2	1	50%
Klongluang	3	2	0	0%	1	0	0%	3	2	67%
Patuwan	3	0	0	0%	3	2	67%	1	1	100%
Patuwan	6	1	1	100%	3	1	33%	5	1	20%
Total	23	14	6	43%	17	6	35%	17	7	41%

Table 2 showed the result of detecting three groups of antibiotic residues, which are Tetracycline, Macrolide, Aminoglycoside, Sulfonamide, and Penicillin, in chicken from fresh markets in Bangkok in a number of 9 samples. It was found that 6 samples of the total (66.67%) detected Tetracycline and 3 of them contain more Tetracycline than MRLs which are 50% of the samples that were tested positive. 8 samples of the total (88.89%) detected Macrolide, Aminoglycoside, and Sulfonamide and 3 of them contain more Macrolide, Aminoglycoside, and Sulfonamide than MRLs which are 38% of the samples that were tested positive. 4 samples of the total (44.44%) detected Penicillin and 1 of them contain more Penicillin than MRLs which are 25% of the samples that were tested positive.

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Table 2. Number of Chicken samples detected drug residues which exceeded MLRs / Range of amount of drug residues detected categorised by sources of the samples (N=9)

No.	Total Chicken Sample	Tetracycline			Macrolide, Aminoglycoside, Sulfonamide			Penicillin		
		Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs
Dindaeng	1	1	0	0%	1	1	100%	1	0	0%
Prawet	1	1	1	100%	1	0	0%	0	0	0%
Bangkapi	2	1	1	100%	1	0	0%	0	0	0%
Patumwan	2	0	0	0%	2	2	100%	0	0	0%
Pomprab Tupai	1	1	1	100%	1	0	0%	1	1	100%
Lamlooka	2	2	0	0%	2	0	0%	2	0	0%
Total	9	6	3	50%	8	3	38%	4	1	25%

Discussion

23 samples of pork bought from different fresh markets in Bangkok were tested to find antibiotic residues in the pork sample. The result showed that 14 samples(60.87%) were detected with Tetracycline in them. Approximately 73.91% were detected with Macrolide, Aminoglycoside, Sulfonamide, and Penicillin. Nevertheless, 43% of the detected samples have a positive result in Tetracycline which has more than the MRLs. 35% of the detected samples have a positive result in Macrolide, Aminoglycoside, and Sulfonamide which is more than the MRLs, and 41% of the detected samples have a positive result in Penicillin which exceeds the MRLs. After analysing the result of detecting antibiotic resistance in 9 samples of chicken, Tetracycline was detected in 6 of the samples(67%). 3 samples(50%) were detected with Tetracycline exceeding the MRLs. Macrolide, Aminoglycoside, and Sulfonamide were detected in 8 samples(88.88%) and 3 samples(37.5%) that exceed the MRLs. Penicillin was detected in 4 samples(44%) and 1 sample(25%) exceeded the MRLs. As a result of detecting antibiotic residue in pork and chicken, it illustrates that some farmers use antibiotics in the wrong way, which results in the exceeding of antibiotic residue that is higher than the standard that the law stated.

By collecting the information during January and February 2565, it relates to the study of Jatesada Jiwakanon[9] that detected antibiotic residue in pork in the area of northeast Thailand from 2560-2561, they detected antibiotic residue in pork(16.6%). Satitkoon Maitreejit[10], who studied antibiotic residue in pork and meat that was sold in fresh market in Phitsanulok, collected samples from July 2562 to October 2563. He detected antibiotic residue at different levels depending on the type of the samples, pork(2.7%) and meat(7.1%). Foundation for consumers, Indy Consumer Magazine randomly collected samples from department stores, fresh markets, and online stores from 9 - 15 June 2561, the number of samples that detected antibiotic residue in chicken and chicken's liver(41.93%)[11] The research and report about the detection of antibiotic residue in pork and chicken during 2560-2563, detected antibiotic residue in pork and chicken. Anong

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Bintwihok and Danis Davitiyananda were finding antimicrobial residue in pork, chicken, and cow's milk in 2003 in the number of 600 samples in total, 200 each sample. He detected Gentamicin(20%), Chlortetracycline(12.5%) in chicken and Tetracycline(40%), Chlortetracycline(20%), Gentamicin(15%), and Oxytetracycline(12.5%) in pork[(2)].

Sunpetch Angkititrakul and et al. conducted a study on Detection of Antibiotic Residues in Pork in Khon Kaen Municipality by Triple Medium Test with Trimethoprim during Jan-May in 1999. 300 pork samples were tested and 14 pork samples (4.67%) were found to contain antibiotic residues and 20 pork samples were suspected to contain antibiotic residues due to the test methods could not indicate the type and quantitative of antibiotic residues in meat[(6)]. Researching antibiotic residue for several years demonstrates that antibiotics are still used with livestock. The detection of antibiotic residue regularly may be because farmers don't use antibiotics with livestock properly, which will cause antibiotic resistance in livestock, antimicrobial resistance, and antibiotic residue in the meat. This is related to the report of The Department of Medical Sciences that has investigated microbial resistance in 3 types of meat which are pork, beef, and chicken. A total of 6 types of bacteria were isolated namely E. coli, E. faecalis, E. faecium, S. aureus, Salmonella spp., and Campylobacter spp. Meats that were found to be most contaminated with bacteria are chicken (91.2%), beef (81.5%), and pork (77.0%). Moreover, the distribution sources that were found to be most contaminated with bacteria are fresh markets (98.5%), modern trades (86.7%), and local brands (56.5%). The highest rate of microbial resistance is found in chicken (78.9%), followed by pork (65.6%), and beef (51.8%)[(14)].

Pork and chicken are the main ingredients in cooking food. As a result of this experiment and the previous research on this topic, antimicrobial resistance and the antibiotic residue was detected. If consumers receive these bacteria and residue into their bodies, the antimicrobial residue would be a problem in the future. The number of people who have antibiotic resistance and the death of people from antibiotic resistance increases each year. Moreover, ingesting safe food is the essential way to stop receiving antibiotic resistance, cook thoroughly and eat meat from farms that do not use antibiotics, which might be another way to reduce the antibiotic resistance from the food that people consume.

Limitation

The limitation may affect the results which cause incomplete information in our experiment. First of all, the information and samples were collected in some of the area in Bangkok. Secondly, the time spend of this experiment was done only one month, which is on February. Thirdly, the test kit that was used may not be accurate as the other type of test. Therefore, the results may not be accurate but almost accurate in terms of human skill.

Conclusion

23 pork and 9 chicken samples from fresh markets were tested to find antibiotic residues; Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin. The result showed that 14 (60.87%) pork samples were detected with Tetracycline in it where 6 sample exceeded MRLs. 17 (73.91%) pork samples were detected with Macrolide, Aminoglycoside, Sulfonamide where 7 samples exceed MRLs., And 17 (73.91%) were found to contain Penicillin in it where 6 pork samples exceeded MRLs. 6 (66.67%) chicken samples were found to have Tetracycline in it which 3 (50%) exceed MRLs. Macrolide, Aminoglycoside, Sulfonamide had been found in 8 (88.89%) chicken samples which 3 (37.5%) exceeded MRLs. And 4 chicken samples were detected with Penicillin which 1 sample exceeded MRLs.

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