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Detection of Antibiotic Residues In Fresh Pork In Sold In Fresh Market In Bangkok Metropolitan Area, Thailand

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Abstract

Background: Antibiotics are medicines that kill bacteria or slow down the bacterial infections. The increasing misuse of antibiotics in animals have led us to the issue of antibiotic resistance. Once consumed by humans, they have multiple negative effects on our body such as carcinogenicity.

Objective: To examine antibiotic residues in pork sold in the local fresh markets and supermarkets.

Study Methods: Antibiotic residue detection kits from the Department of Medical Sciences, Thailand which were produced by Rodejanarug Pharmaceutical, were used to detect different types of antibiotic residue (i.e., Penicillins, Amoxicillin, Tetracyclines, Oxytetracycline, Chlortetracycline, Gentamicin, Neomycin, Streptomycin, Sulfadimethoxine, Thyroxin, Erythromycin and Bacitracin) and their concentrations in the samples. These detection kits have 93% accuracy, 78.9% sensitivity, and 96.7% specificity

Results: From a total of 62 pork samples which were randomly brought from different markets in Bangkok, 49 (79.32%) samples were detected with antibiotic residue in it. Tetracycline was found in 45 (73.77%) samples which 6 (13%) samples were exceed MRLs, Macrolide, Aminoglycoside, Sulfonamide was found in 44 samples (72.13%) where 7(16%) samples were exceed MRLs and Penicillin was detected with 24 (39.34%) samples which 2 (8%) samples were exceed MRLs.

Conclusion: Antibiotic residues were detected in 79.6% (45/62) of pork samples which 13% (6) exceeded MRLs.

Keywords: food safety, antibiotic residues, fresh pork

Introduction

Antibiotics are medicines that fight against and slow down bacterial infections in both humans and animals[1]. As the use of antibiotics increased throughout the years, the use of antibiotics from humans was transferred to animals. Now, in the United States, around 70% of antibiotics sold are now used in animals[2]. Antibiotics are used in animals as a growth promoter, a cure for various illnesses, and to prevent them from having infections. However, this led to the problem of having antibiotic residues in our own food. When antibiotics are not used and checked properly, they may end up in food products such as eggs, fish, and meat[3]. Overuse of antibiotics in animals will cause certain bacteria to be resistant to specific antibiotics. Once humans consume meat, there is a chance that we also consume the antibiotic residues and antibiotic-resistant bacteria that the meat previously had. The consumption of antibiotic residues can cause hypersensitivity reaction,

carcinogenicity, mutagenicity, teratogenicity, bone marrow depression, and disruption of normal intestinal flora[4]. Furthermore, the consumption of antibiotic resistant bacteria will lead your body to be resistant to certain antibacterial. As a result, antibiotic resistant bacteria are responsible for more than 2 million illnesses in the US in 2013[5]. This causes additional problems to citizens such as an increase in medical expenses, increased recovery time from infections, and possibly death.

In 2020, according to the Journal of the Medical Association of Thailand, it was found that fresh raw foods, including food from animal products, seafoods, vegetables, fruits and honey from two large wholesale markets in Thailand were contaminated with antibiotic-resistant bacteria and some contained antibiotic residues. Food samples were cultured for antibiotic-resistant bacteria and tested for the presence and amount of antibiotic residue. Among 521 samples for bacterial culture, 86.9% grew at least one kind of bacteria. Among 501 samples for antibiotic residue testing, 37.1% contained at least one antibiotic residue. A Enrofloxacin was the most prevalent antibiotic residue, followed by Doxycycline and Tilmicosin. Although most samples contained less antibiotics than the maximum residue limit (MRL), 7.0% contained an amount of at least one antibiotic above MRL. Therefore, Thai people are at risk of being colonized with antibiotic-resistant bacteria and developing antibiotic-resistant bacterial infection due to consuming foods contaminated with antibiotic-resistant bacteria or containing antibiotic residues. From the department of medical science report for Food Safety detect antibiotic resistance bacteria in chicken meat 78.9%, pork 65.6%, beef 51.8%. this shows that not only antibiotic residue is found in meat but also the high rate of antibiotic resistance[6].

Thus, the objective of this experiment is to determine the types and level of antibiotics in pork from different fresh markets in Bangkok, Thailand.

Objective:

To examine antibiotic residues in Pork sold in fresh market

Instrument and Tools

Sampling

This research focuses on detecting antibiotic residue in fresh pork sold in supermarkets in Bangkok metropolitan area. We use a random sampling method to buy fresh pork from different supermarkets located in different areas in Bangkok. 189 samples were bought twice from 11 different supermarkets from different districts 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 Penicillin, Amoxicillin, Tetracyclines, Oxytetracycline, Chlortetracycline, Gentamicin, Neomycin, Streptomycin, Sulfadimethoxine, Thyroxin, Erythromycin and Bacitracin[7].

Procedure

Storing and preparing meat samples[7]

1. Sampling pork that has been ground for 5 gram each, then put it in a centrifuge tube. (The size has to be 30 millilitres or more, based on type of antibiotic residues.)
2. Add the extract solution A (tetracycline group), extract solution B (macrolide group, aminoglycoside, and sulfonamide group), and extract solution C (penicillin group) for each 5 millilitres.
3. Shake by hand or with a shaker for 10 minutes, then bring to heat in a water bath at 60 degrees Celsius for 5 minutes and make the samples cool down.
4. Centrifuge at 3,000 to 4,000 rounds for 15 minutes to get the transparent part of the samples.
5. Adjust pH of the transparent part for testing.
6. Adjust pH of the negative control sample to make a comparison with the sample.
7. If the original pH is according to the specification, then there is no need to adjust the ph.

- Procedure for testing meat sample

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1. Drop 4 drops of the meat sample that was extracted followed by the desired drug group using the dropper (no bubbles) in each testing set. In the case of finding sulfonamide, use the testing set that contains trimetoprim.
2. Drop 4 drops of the negative control sample into one test tube.
3. Gather the testing set from step 1 and 2 to inoculate the samples in water bath / incubator at the temperature of 64 ± 2˚C. If shrimp and fish, leave in for ≥ 2 hr 30 min. If chicken, pork, or beef, leave it in for ≥ 2 hr. 45 min or read the results from the test tube by giving food under the water level until the negative control sample changes color from purple to yellow.

- How to read and interpret results
    1. If the whole test tube changes colour from purple to yellow = no residues
    2. If the test tube partly changes colour or doesn't change colour = residue is present.
- Efficiency of the testing set
    - This testing set has an accuracy of 93%, a sensitivity of 78.9%, a specificity rate of 96.7%, and has the ability to detect at least 12 different types of drug residues. The testing set can detect the amount of drug residues at the level acceptable according to international standards. Drugs include penicillin, amoxicillin, tetracycline, oxytetracycline, chlortetracycline, gentamicin, neomycin, strep, tomycin, sulfadimethoxine, tyrosine, erythromycin and bacitracin.
- Shelf life of test sample
    - Store the test kit at a refrigerated temperature of approximately 4-8˚C. Everything can be stored for 3 months except for the examination test kit. Sulfonamide antimicrobials stored not more than 20 days.
- After experiment
    - Pour disinfectant solution into test tubes to cover the foods and leave for 30 mins or boil test tubes in water with lid for 15 mins or use the autoclave and trash the tubes afterwards.

Results

From the inspection remaining antibiotics from 62 samples of fresh pork from supermarket, it can be seen that there are total of 49 samples from 62 that contains remaining antibiotics which can be calculated as 79.32% (Table 1)

Table 1. Percentage of Pork sample detected antibiotic residues (Tetracycline, Macrolide, Aminoglycoside, Sulfonamide, Penicillin) (N=62)

No. of sample	No. of sample detected drug residue	Percentage of sample detected
62	49	79.32

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From the experiment conducted to test antibiotic residue by 3 types of drugs in the 62 samples, found that, First, Tetracycline type antibiotics were detected in 45 samples of pork which is calculated to be 73.77%. Second, Macrolide, Aminoglycoside, Sulfonamide type antibiotics were detected in 44 samples of pork which is 72.13%. Third, Penicillin was detected in 24 of the samples which is 39.34% (Table 2)

Table 2. No. of Pork sample detected antibiotic residues and percentage of sample detected antibiotic residues categorised by the source of the samples. (N=62)

Source	Total Pork Sample	Tetracycline	Macrolide, Aminoglycoside, Sulfonamide	Penicillin
		Positive	Positive	Positive
Supermarket 1	8	4	4	2
Supermarket 2	6	5	5	1
Supermarket 3	11	6	6	4
Supermarket 4	7	5	5	3
Fresh Market	4	4	4	3
Supermarket 5	6	6	5	3
Supermarket 6	7	6	5	2
Supermarket 7	2	1	0	0
Supermarket 8	3	1	2	3
Supermarket 9	2	2	2	2
Supermarket 10	6	5	6	1
Total	62	45	44	24
% Of sample detected		73.77%	72.13%	39.34%

According to the test results for Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin antibiotic residue from 62 fresh pork samples, Tetracycline antibiotic residue was found in 45 samples of fresh pork and 6 of them exceeded MRLs which are 13% of the samples that were tested positive. 44 samples were tested positive for Macrolide, Aminoglycoside and Sulfonamide antibiotic residue and 7 samples from those positive results exceeded MRLs which are 16% of the positive tested samples for this group. And Penicillin antibiotic residue was found in 24 samples of the fresh pork and 2 of them exceeded MRLs which are 8% of the positive tested samples. (Table 3)

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Table 3. Number of Pork sample detected antibiotic residues which exceeded MRLs, and percentage of sample detected antibiotic residues which exceeded MRLs categorised by sources of the samples. (N=62)

Source	Total Pork Sample	Tetracycline			Macrolide, Aminoglycoside, Sulfonamide			Penicillin		
		Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs	Positive	Positive >MRLs	% >MRLs
Supermarket 1	8	4	1	25%	4	1	25%	2	1	50%
Supermarket 2	6	5	1	20%	5	1	20%	1	0	0%
Supermarket 3	11	6	0	0%	6	0	0%	4	0	0%
Supermarket 4	7	5	0	0%	5	0	0%	3	0	0%
Fresh Market	4	4	1	25%	4	2	50%	3	0	0%
Supermarket 5	6	6	2	33%	5	1	20%	3	1	33%
Supermarket 6	7	6	0	0%	5	0	0%	2	0	0%
Supermarket 7	2	1	0	0%	0	0	0%	0	0	0%
Supermarket 8	3	1	0	0%	2	0	0%	3	0	0%
Supermarket 9	2	2	1	50%	2	0	0%	2	0	0%
Supermarket 10	6	5	0	0%	6	2	33%	1	0	0%
Total	62	45	6	13%	44	7	16%	24	2	8%

Discussion

From the results of this experiment, 49 out of 62 samples, or 79.32% of all samples, contained antibiotic residues. These samples had percentage residues of Tetracycline, Macrolide, Aminoglycoside, Sulfonamide, and Penicillin of 73.77%, 72.13% 39.34%, respectively. However only 13%, 8%, 16% of Tetracycline, penicillin and Macrolide, Aminoglycoside, Sulfonamide, respectively, were detected to have residue more than MRLs.

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According to a study by Natthida Suksai and her team, there have been an increase in the misuse of antibiotics in animal farms every year[8] which leads to antibiotic resistance. They found that Chiang Mai farmers used a huge amount of antibiotics in their animal ranches while still having the misunderstanding of how to appropriately use antibiotics.

The results from this study are similar to the study of Anong Binthavihok and Danit Thaveitiyanon(2545); they examined antimicrobial residues in chicken, pork, and cow dairy 200 samples each which is summed to 600 examples. Later it was found that in chicken there are 20% of them contains gentamicin and 12.5% contains chlortetracycline. While in pork, there was 40% of them contains tetracycline, 20% contains chlortetracycline, 15% gentamicin, 12.5% oxytetracycline[9]. In addition, a research team investigated the remaining antibiotics in 300 samples of pork in Khon Kaen by using TMT (Triple medium test with Trimethoprim) method (2545) as screening due to its cost and pace. However, the test cannot indicate the type of antibiotic residues in pork. After running 300 samples, there were 14 samples (4.67%) that contained antibiotics with another 20 samples (6.67%) being suspected[10]. The Department of Medical Sciences investigated the antibiotic residue in pork, chicken, beef, and shrimps during 2003-2005. From a total 571 samples, 83 samples were found to contain antibiotic residues[11]. Moreover, the report of Integrated Food Safety, Department of Medical Sciences, found that 78.9%, 65.6%, 51.8% of chicken, pork and beef, respectively, contained antibiotic residues[12].

From the analysis of this research along with other studies, it can be predicted that the number of patients that have antibiotic resistance will increase every year[13]. Antibiotic residue in the meat could develop into antibiotic resistance in animals and if these meats were consumed unhygienically, it could lead to health problems in the future. This research founded that consumers are risk of food safety in many perspectives including antibiotic residue in meat[14]. Consumers should have understanding in the safety of food and how to choose food and process food safely and safe from health problems that are caused by contaminated food both in the short term and long term.

Limitation

This research was based on the examination of pork samples that were bought randomly from Bangkok supermarkets during January to February 2022 only. During the research project, there was an outbreak of African Swine Fever which could make farmers and the related agencies be more conscious and stricter in the pork qualities than any other time without the outbreak. It might not be concluded that the result was the overall Thailand's result during other periods of time.

For the testing process, the antibiotics test kits were used, thus, the results could be slightly different from the Laboratory's result.

Conclusion

From a total of 62 pork samples which were randomly brought from different markets in Bangkok, 49 (79.32%) samples were detected with antibiotic residue in it. Tetracycline was found in 45 (73.77%) samples which 6 (13%) samples were exceed MRLs, Macrolide, Aminoglycoside, Sulfonamide was found in 44 samples (72.13%) where 7(16%) samples exceed MRLs, and Penicillin was detected with 24 (39.34%) samples which 2 (8%) samples were exceed MRLs.

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