Situation of Antibiotic residues in fresh meats and ready to drink cow milk sold for consumers in Bangkok

Pitchapat Thanapatcharoen[1],Pasinee Phuhoy[2] ,Piyalada Sonroi[3] ,Oatthaya Kongsinkaew[4] ,Parit Kosonsasitorn[5] ,Napat Charoensin[5] ,Pariyachat Somyarach[6], Napatsanan Champati[7] , Nicharat Akkhararattanawit[8], Sirawit Payomhom[9], Wimolthip Kaowthongborisut[10], Chatchaya Chalongnantachai[10], Chayanat Chalongnantachai[10]

[1]Patumwan Demonstration School, [2]Rajavinit Mathayom School, [3]Sarasas Witaed Nakhonratchasima, [4]Boonwattana School, [5]Montfort College, [6]Samakkhi Witthayakhom School, [7]Satree Prasertsin School , [8]Satriwittaya School, [9] Chalermkwansatree School, [10]Sarasas Witaed Suvarnabhumi School
Corresponding Author: Pitchapat Thanapatcharoen, email: petch254731@gmail.com

Abstract
Background: Irrational antibiotic uses in livestock resulting in antibiotic residues in pork, chicken, meat and other products from animals.
Objective: to examine antibiotic residues in pork, chicken, meat, and ready-to-drink cow milk
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. Convenience sampling was conducted. A total of 263 samples which consisted of 115 samples of fresh pork, 21 samples of chicken and 12 samples of fresh beef sold in the fresh markets, 43 samples of ready-to-eat cow's milk, 36 samples which advertised as organic pork, and 36 samples which advertised as hygienic chicken were tested for antibiotic residues; Tetracycline, Macrolide, Aminoglycoside, Sulfonamide, Penicillin.
Result: From a total of 263 samples, the results showed that 148 sample of fresh pork, chicken and meat bought from fresh market were found antibiotic residues in 103 samples (69.59%); fresh meat (n=12, 100%), fresh chicken (n=18, 85.71%) and fresh pork (n=73, 63.49%). Results of ready-to-drink cow milk showed that from a total of 43 samples, 6 samples (13.95%) were found to contain Tetracycline residues. 72 samples of fresh pork and chicken, 36 each, which advertised as hygienic were examined, no sample was detected of Tetracycline residue
Conclusion: 69.59% (103/148)of fresh pork, chicken and meat samples and 13.95% (6/43) of ready-to-drink cow milk samples were detected antibiotic residues. No antibiotic residues found in 72 samples consisted of 63 samples of fresh pork and 36 samples of chicken which advertised as hygienic.
Keyword: drug residues, antibiotic residues, drug resistance, food safety

Introduction

Antimicrobial is the most commonly pharmaceutical used to destroy or inhibit the growth of pathogenic microorganisms in humans, animals and plants in raising economic animals to produce meat, which is an essential protein source for the body. Antimicrobial agents are used regularly. Its purpose is to prevent, treat diseases and to stimulate animal growth (1) but sometimes animal illness may not be related to infection or a type of infection that does not respond to the drug of choice. As a result, the use of antimicrobial drugs is not successful in treating consumers. In addition, farmers prefer to use antimicrobials to prevent disease in animals as well as used as a supplement in animal feed (food additives) to accelerate growth. Inappropriate antimicrobial usage or incorrect, such as giving too low a dose for a long period of time giving a higher dose than the standard prescribed, not giving the full dose of medication, stop the medication too soon or not stop the medication before sending it to the slaughterhouse. These are the causes of antimicrobial residues in the tissues [2] of edible animals, from meat, organs and organs, including milk and eggs. This may cause adverse effects on the health of consumers, such as causing allergic reactions to drugs, pathogens in the body that are resistant to drugs and some are carcinogenic, etc. [3–5]. Antimicrobial in meat. As a result, consumers are inevitably affected by the following: allergic reaction, especially penicillin antibiotics; of antibiotics, affects the health of many consumers who believe that about 1-10% of the people in the world are allergic to this type of antibiotic. As for antibiotics in the Aminoglycosides group cause allergic reactions comparable to penicillin. [6] There is also an antibiotic that has been found to be highly allergic to consumers, streptomycin, which is neurotoxic and sulfonic. Sulfonamides cause anemia [7]. The problem of microbial resistance in both animals and humans is also important. As a result, the treatment of the disease is not as effective as it should be because microorganisms will not be destroyed when given the same amount of drug. In particular, the enteric group of bacteria and the Staphylococcus group. Some antibiotics have also been found to have cancer-inducing activity, such as Nitro furans. Chloramphenicol causes non plastic anemia. Therefore, chloramphenicol is contraindicated in edible animals [9].

Currently, the animal industry for consumption to expand rapidly. Therefore, in addition to the various technologies that manufacturers use in the animal industry process. There are also drugs and chemicals to accelerate growth maximize productivity. One of the most widely used drugs is antimicrobial includes antibiotics. Most of the Antimicrobial used in animals is the same class of drugs used in humans. Therefore, the use of such drugs is incorrect or unreasonable. Thus, affecting both the animals, the owners, consumers and the environment causing a major problem that has a high impact around the world. The problem of drug-resistant bacteria is an important problem in public health which caused enormous damage in terms of health and the country's economy [10]

African swine fever (African swine fever; ASF) is an epidemic that continues to spread in regions around the world. This has serious consequences in swine family, including domestic pigs and wild boars [11]. The disease is often so severe that almost 100% of the infected pigs die acutely. The environment is high, recovered pigs can be carriers of the disease for life (Carrier) because the infection is still latent (Persistent infection) making it difficult to get rid of the disease once the disease has occurred in the country. At present, there is no vaccine to prevent disease and studies are still being conducted to develop a vaccine. The outbreak of the epidemic has a direct impact on the economy and high society. Although, this infection is an infection that does not infect people. (Non-zoonosis), however, the theoretically assessable damage from ASF is caused by death of pigs on farms, disease control and loss of ability to export swine products to the world market are primarily for Thailand, which may affect the drug behavior of farmers in livestock. Inappropriate usage of antibiotics affects drug residues in the meat that consumers eat and may affect the problem of drug resistance. Therefore, this research aims to investigate the prevalence of antibiotic residues in animal products used as food for human consumption in Bangkok which is an area with many inhabitants.

Microbial resistance is caused by microorganisms that develop their ability to withstand the antimicrobials used by people. The problems that follow will make the treatment of the infection ineffective. At present, the situation of antimicrobial resistance in the world and in Thailand tends to worsen. It was found that the number of drug-resistant microorganisms increased by antimicrobial research or new antibiotics to address drug-resistant bacteria are insufficient and do not keep up with demand. This makes it difficult to treat patients with drug-resistant infections, high cost, toxic and side effects and causing death. The reason for the resistant pathogens is a matter of nature caused by the evolution of microorganisms for their own survival. Antimicrobial survivors have created new genes known as “drug resistance genes. The evolution of drug resistance is accelerated if the drug is overused significantly. [12]

Microbial resistance is one of the biggest public health security challenges of the 21st century. Antimicrobials that were once magic drugs in the 20th century are steadily weakening due to their wide use in humans and animals until many types of germs became resistant to drugs cause in each bedridden It takes longer to heal. World Health Organization (WHO) has issued a warning against antibiotic misuse by doctors and said the last-generation antibiotic was no longer effective. While each country's science ministers who attended last year's G8 summit acknowledged that the disease's resistance to pathogens. It has become a major public health challenge in the 21st century. The meat industry uses more antibiotics than necessary so that make many parties whether governments, private companies and consumers are concerned that this will make the germs immune to the drug. As a result, medical treatment takes longer which is detrimental to humans because there are more residues in the body as well [13]

Study Objective

1. To study the prevalence of antibiotic residues in fresh pork, fresh chicken, fresh beef sold for consumers.

2. To study the prevalence of antibiotic residues in ready-to-drink cow's milk sold for consumers.

3. To study the prevalence of antibiotic residues in fresh pork, fresh chicken, and fresh beef advertised as hygienic that are sold for consumers.

Instrument and tools

Population and Sampling

This study examined the group of antibiotic residues; Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin in samples of fresh pork, fresh chicken, fresh beef, ready-to-drink cow's milk and fresh pork, fresh chicken advertised as non-toxic (Hygienic Pork, Hygienic Chicken) from the Bangkok area. Convenience sampling was conducted by using 115 samples of fresh pork, 21 samples of chicken and 12 samples of fresh beef sold in the fresh markets, 43 samples of ready-to-eat cow's milk, 36 samples of organic pork, and 36 samples of organic chicken meat, a total of 263 samples sold in Bangkok.

For the detection of Antibiotic Residues in fresh pork samples and fresh chicken, the researcher used the Antibiotic Residue Detection Kit of the Department of Medical Sciences produced by Rojanarak Pharmacy. This test kit has an accuracy of 93%, a sensitivity of 78.9%, a specificity of 96.7%, and ability to detect at least 12 drug residues. International standards are Penicillin, Amoxicillin, Tetracycline, Oxytetracycline,

Chlortetracycline, Gentamycin, Neomycin, Streptomycin, Sulfadimethoxin, Tyrosin, Erythomycin, and Bacitracin [14].

The antibiotic test kit uses Bacillus Stearothermophilus spores as a tester by producing bacteria at a concentration of 108 CFU/ml. in a culture media that is suitable and favorable for penetration of antibodies. Microorganisms and spore growth are contained in plastic tubes. (Polypropylene) size 1 cm in diameter, 4 cm in height and put a color indicating the acidity – alkalinity. Normal test kits are purple (pH=6.8). If acidity occurs in the test kit, it turns yellow (pH = 4). When testing, drop 0.1 ml. of the sample into test kit [14].

Reading the results of the Antimicrobial residual test in the meat sample by looking at the color change in the test tube, if the test tube color changes to yellow, the sample shows no microbial residue, but if the test tube color remains purple, it means there is microbial residue. If the color of the test tube is purple and has a yellow underside or a lighter purple but not yellow, the sample may contain low amounts of microbial residues by the concentration that the probe can detect (Detection limits) can be 100% because the principle of the check set relies on the division of B. Stearothermophilus in case it is not wrinkled. Microbial medicine then takes the nutrients in a test tube and creates a corticosteroid state. This causes Bromcresol Purple (purple) to change from purple to yellow, depending on the acidic conditions in the test tube and the reason for choosing B Stearothermophihus because B Stearothermophilus Navratilova (Navratilova, Navratilova, Navratilova, Navratilova) is a viable candidate for rapid growth at high temperatures (64°C) and is highly sensitive to the detection of β-lactam antibiotics in livestock (Navratilova). B.,D. Janstova Vorlova, 2008. Occurrence of Tetracycline Chlortetracyclin and Oxytetracycline Residues in Raw Cow's Milk. Czech J. Food Sci. Vol. 27, 2009, (5) 379-385)

Study Material

The equipment uses to test for antibiotic residues in fresh pork and fresh chicken are as follows:

1) 50 finished test tubes
2) 10 plastic dropper tubes
3) Equipment used for the inspection
4) Hot tub /Incubator (temperature control 64±2˚C)
5) Liquid Extract A, B and C (choose the extract according to the type of drug residue to be examined)

Testing for Antibiotic residues in Fresh Pork and Fresh Chicken (Test Procedure)

Process of collecting and preparing meat samples

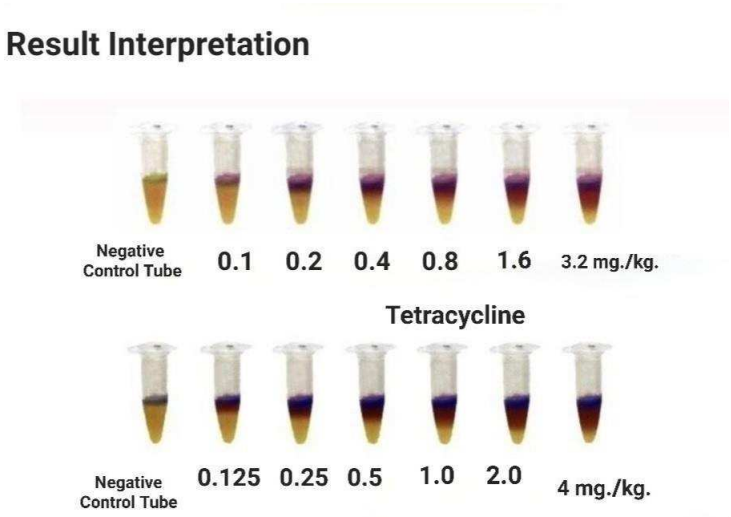
1. Chop or grind the animal samples thoroughly.
2. Sampling 15 g. of minced meat into 30 ml. in 3 centrifuge tubes, divided into 5 g. each tube.
3. Add extract A (Tetracycline group), extract B (Macrolide, Aminoglycoside and Sulfonamide) and extract C (Penicillin group) 5 ml. in each centrifuge tube.
4. Shake the tube well and wait for 1 hour and 45 minutes by feeding the culture in the tube at the water level until the negative control sample tube completely changes color from purple to yellow.

Then observe the color of the sample tube and interpret the result.

Result Interpretation

Take the sample tube and compare the color compared with the negative control sample.

1. If the test kit turns yellow throughout the tube, it indicates that no antibiotic residue was found.
2. If the test kit turns purple Purple-yellow or purple whole tube, the color bands depend on the amount of antibiotic residues in the meat [14].



Result

From the antibiotic residues in the samples, 148 samples were tested, of which 115 were pork, 21 were chicken, and 12 were beef. The percentages were as follows: 115 pork, 71 were found Tetracycline representing 61.74%. Macrolide, Aminoglycoside, Sulfonamide were found in 73 specimens representing for 63.48% and were found Penicillin in 53 specimens, representing for 46.09%. Chicken 21 specimens were found Tetracycline in18 specimens representing for 85.71%. Macrolide, Aminoglycoside, Sulfonamide were found in 18 specimens representing for 85.71% and Penicillin was found in 16 specimens representing 76.20% and 12 meat samples, Tetracycline in 12 samples, or 100% were found. Macrolide, Aminoglycoside, Sulfonamide in 12 samples, or 100% were found and Penicillin 12 samples, or 100% were found, the residues were found the most are Macrolide, Aminoglycoside, Sulfonamide in 103 samples representing 69.60%, Tetracycline in 101 samples representing 68.24% and Penicillin was found in 81samples representing 54.72%, respectively (Table 1).

Table 1. No. of sample that detected antibiotic residues and percentage of sample detected antibiotic residues categorized by the type of the samples.

Type	**Total Sample**	**Tetracycline**	**Macrolide, Aminoglycoside, Sulfonamide**	**Penicillin**
		Positive	**Positive**	**Positive**
Pork	115	71	73	53
Chicken	21	18	18	16
Meat	12	12	12	12
Total	**148**	**101**	**103**	**81**

From 115 samples of pork, Antibiotic residues were found in 73 samples, 21 samples of chickens, 18 samples were found antibiotic residues, 12 samples of meat, 12 samples were found antibiotic residues, a total of 148 samples were found antibiotic residues in meat the most, reached 100%, chicken 85.71% and pork 63.49%, respectively (Table 2).

Table 2. Percentage of sample detected antibiotic residues and percentage of sample detected antibiotic residues categorized by the type of the samples

Type	**Total Sample**	**No. of sample that detect Antibiotic Residues**	**Percentage of antibiotic residues detected**
Pork	115	73	63.49
Chicken	21	18	85.71
Meat	12	12	100.00
Total	**148**	**103**	**69.59**

From the table, cow milk 43 samples were found antibiotic residues in 6 samples, representing for 13.95%. (Table 3)

Table 3. No. of sample and percentage of Cow Milk that detected antibiotic residues

Type	**Total Sample**	**Tetracycline**	
		Positive	**Percentage of Antibiotic residues**
Cow Milk	**43**	**6**	**13.95**

From the hygienic samples were taken for antibiotic residues, out of the 36 pork samples and 36 chicken meat samples, it was found that residues of Tetracycline is 0 sample Macrolide, Aminoglycoside, Sulfonamide 0 sample, Penicillin 0 sample, No antibiotic residues were found in the samples. Therefore, hygienic meats are highly safe from antibiotic residues (Table 4).

Table 4. No. of hygienic sample that detected antibiotic residues and percentage of sample detected antibiotic residues categorized by the type of the samples

No.	**Type of Meat**	**Total sample**	**Number of Sample that detected residues**		
			Tetracycline	**Macrolide Aminoglycoside, Sulfonamide**	**Penicillin**
1	Fresh Pork	36	0	0	0
2	Fresh Chicken	36	0	0	0
	Total	**72**	**0**	**0**	**0**

From the hygienic samples were taken for antibiotic residues the out of 36 pork samples and 36 chicken samples, residues of Tetracycline was found 0%, Macrolide, Aminoglycoside, Sulfonamide 0%, Penicillin 0%. Therefore, hygienic meats are highly safe from antibiotic residues (Table 5).

Table 5. Percentage of Hygienic sample that detected antibiotic residues and percentage of sample that detected antibiotic residues categorized by the type of the samples

Type	Total Sample	No. of sample that detected Antibiotic Residues	Percentage of antibiotic residues detected
Fresh Pork	36	0	0%
Fresh Chicken	36	0	0%
Total	**72**	**0**	**0%**

Discussion

This study examined antibiotic residues (Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin) in a total of 263 samples which consisted of 115 fresh pork samples, 21 fresh chicken samples, 12 fresh meat samples, 36 samples of fresh pork advertised as hygiene, and 36 samples of chicken advertised as hygiene. The result showed that 148 samples of fresh pork, chicken and meat bought from fresh market were found antibiotic residues in 103 samples (69.59%); fresh meat (n=12, 100%) , fresh chicken (n=18, 85.71%) and fresh pork (n=73, 63.49%). These findings were in line with several previous studies [15][16]; food safety report conducted in 2019 that antimicrobial resistance bacteria were found in 78.9% of fresh chicken samples, 65.6% of fresh pork samples and 51.8% of fresh meat samples [17]. A study conducted in 2002, examined antibiotic residues in both fresh chicken and fresh pork samples of 200 each from Bangkok and vicinities, Chonburi and Nakhon Pathom province and found antibiotic residues in these [18]. There were previous studies conducted in Khon Kaen province by Sunpetch Angkititrakul and et al. tested fresh pork sold in the municipality area and found antibiotic residues in pork samples (n=14, 4.67%). Duangdao Wongsommart and et al. examined 571 samples consisting of fresh pork, fresh chicken, fresh meat and fresh shrimp samples and found antimicrobial resistance residues in 83 samples (14.5%). For ready-to-drink cow milk, from a total of 43 samples, 6 samples (13.95%) were found Tetracycline residues. This is due to most livestock in Thailand used antibiotics with their animals and there were also some irrational use of antibiotic in livestock. Therefore; it's consequent of residues in fresh pork, fresh chicken, fresh meat and ready-to-eat cow milk when it came to consumers.

72 samples of fresh pork and chicken, 36 each, which advertised as hygienic were examined, no sample was detected of Tetracycline residue. This is because farms which can produce hygienic pork must be certified farm standard by the Livestock Department of Thailand. The farm must have a qualified system to control plague in animals as well as being qualified for standard of farm management. Pigs need to be blood and urine tested for Leanness-enhancing agents and antibiotic residues before being produced into pork [19].

This study revealed that fresh pork, fresh chicken and fresh meat which were traditionally raised in common farms had a higher risk of containing antibiotic residues. While pork and chicken which were advertised as hygienic where being produced by certified farms and governed by systematic processes showed to be antibiotic free. Thus; one of the ways to avoid receiving antibiotic resistance bacteria from animal product consumption is to choose hygienic meat products for consumption.

Limitation

The antibiotics residue test kits were used to test Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin residues in this study. Therefore; the result could be slightly different from the laboratory's result. During the study period, there was an outbreak in swine. For this reason, it could lead to a more usage of drugs in swine farms to prevent the plague.

Conclusion

Antibiotic residues (Tetracycline, Macrolide, Aminoglycoside, Sulfonamide and Penicillin) were tested for 263 samples which consisted of 115 fresh pork samples, 21 fresh chicken samples, 12 fresh meat samples, 36 fresh pork advertised as hygiene, and 36 samples of chicken advertised as hygiene. The results showed that 148 sample of fresh pork, chicken and meat bought from fresh market were found antibiotic residues in 103 samples (69.59%); fresh meat (n=12, 100%), fresh chicken (n=18, 85.71%) and fresh pork (n=73, 63.49%). Results of ready-to-drink cow milk, showed that from a total of 43 samples, 6 samples (13.95%) were found to contain Tetracycline residues. 72 samples of fresh pork and chicken, 36 each, which advertised as hygienic were examined, no sample was detected of Tetracycline residue.

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