Original Article

Heat Stability of Antibiotics Commonly Used in Food Animals and Agriculture in Thailand

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Objective: To determine the heat stability of antibiotics commonly used in food animals and agriculture in Thailand.

Materials and Methods: Seventeen commonly used antibiotic formulations for food animals and agriculture in Thailand were included. The antimicrobial activity of the tested antibiotics was determined by the minimum inhibitory concentration (MIC) of the antibiotics against *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *B. subtilis* ATCC 6633. The MIC of each antibiotic against each bacterium was performed by the broth microdilution method. The MICs of untreated antibiotic samples and heat-treated antibiotic samples after exposure to heat at 100°C for 30 minutes and/or after heating at 121°C for 15 minutes were determined. A heat-stable antibiotic was defined as one for which the MIC of the heat-treated antibiotic was not more than twofold the MIC of the untreated antibiotic. A heat-labile antibiotic was defined as one for which the MIC of the heat-treated antibiotic was more than twofold the MIC of the untreated antibiotic.

Results: Bacitracin and neomycin sulphate were heat-labile at 100°C and 121°C, whereas amoxicillin and amoxicillin/clavulanate seemed to be heat-stable at 100°C but were heat-labile at 121°C. The MICs of heated tetracycline samples at 121°C were two- to fourfold the MICS of untreated tetracycline samples. Enrofloxacin, gentamicin, kanamycin, tiamulin, tilmicosin, tylvalosin and colistin were quite stable to heat at 121°C.

Conclusion: Many antibiotics commonly used in food animals and agriculture in Thailand are heat-stable. Therefore, ingestion of heat-cooked foods containing heat-stable antibiotic residues could still present a threat for the emergence of antibiotic-resistant bacteria in humans.

Keywords: Heat Stability, Antimicrobial Agents, Food Animals, Agriculture

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Antimicrobial resistance (AMR) is a major public health problem worldwide. The annual AMR burden in Thailand is estimated to account for 100,000 new AMR infections, an additional 3 million days of hospitalization, and 30,000 deaths[1]. The annual cost of AMR infections in Thailand is estimated at US$200 million for the costs of antibiotics for treatment, and US$13,000 million, or 0.6% of GDP, for the total economic loss[1]. It is projected that there will be 10 million AMR-related deaths each year and a 3% annual reduction in world GDP by 2050 if containment of AMR at a global level is not achieved[2]. Therefore, the World Health Organization (WHO) has launched a global action plan for AMR since 2015[3]. The plan represents a tripartite collaboration among WHO, the World Organisation for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO).

The most important driver of AMR is the usage of antibiotics in humans, food animals, companion animals and agriculture, especially when antibiotics are inappropriately overused. Antibiotics consumed by humans and animals could induce susceptible bacteria residing in humans and animals to become resistant bacteria; these may colonize and cause AMR infections in humans and animals. Moreover, AMR bacteria induced by antibiotics from humans and animals can be transmitted to humans, the food chain

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and the environment. In addition to the induction of AMR bacteria, the inappropriate use of antibiotics in food animals and agriculture can lead to the presence of antibiotic residues in their products. Antibiotic residues have been detected in many food items, including meat, eggs and seafood[4,5]. Some antibiotic residues have been detected in food sold at open markets in Thailand, such as ampicillin in oranges; and ampicillin, enrofloxacin, chlortetracycline, oxytetracycline, doxycycline, and tilmicosin in fresh pork, fresh chicken and shrimp. Similar to the direct consumption of antibiotics for the treatment and prevention of infections, the consumption of raw food containing antibiotic residues is hazardous to humans as a result of the induction of AMR. However, much food is cooked with heat prior to consumption. Therefore, some heat-labile antibiotics could be inactivated by appropriate cooking, and the foods containing heat-labile antibiotic residues might be unable to induce AMR. On the other hand, heat-stable antibiotics are still hazardous to humans who consume the foods containing heat-stable antibiotic residues, despite having been properly cooked with heat.

The objective of the study was to determine the heat stability of antibiotics commonly used in food animals and agriculture in Thailand.

Materials and Methods

Tested Antibiotics

Seventeen tested antibiotic formulations were purchased from veterinary drug stores or food animal farms. They were finished products for use in food animals, and antibiotic powder preparations for mixing with the feed for pigs. The finished products were 1) amoxicillin 50 mg and clavulanate 12.5 mg per tablet, 2) amoxicillin 100 mg and clavulanate 25 mg per tablet, 3) bacitracin 75 IU and neomycin sulphate 2.5 mg, 4) enrofloxacin 50 mg/mL, 5) gentamicin 40 mg/mL, 6) kanamycin 25 g/100 mL, and 7) oxytetracycline 50 mg/mL. The antibiotic powder preparations were 1) amoxicillin 50%, 2) colistin 40%, 3) chlortetracycline 20%, 4) doxycycline 10%, 5) enrofloxacin 50%, 6) tiamulin H. fumarate 10%, 7) tiamulin 25%, 8) tilmicosin phosphate 20%, 9) tylvalosin 62.5%, and 10) enrofloxacin 20%. Fresh stock solutions of the tested antibiotics were prepared by diluting or dissolving each antibiotic with sterile distilled water.

Tested Bacteria

The tested bacteria were *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *B. subtilis* ATCC 6633.

Antibiotic Susceptibility Test

The antimicrobial activity of the tested antibiotics was determined by the minimum inhibitory concentration (MIC), which was the lowest concentration of antibiotic that inhibited visible growth of bacteria. The MIC of each antibiotic against each tested bacteria was determined by the standard broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI M100, 2017). Cation Mueller-Hinton broth (BBL, Becton Dickinson, USA) was used to dilute the untreated or heat-treated antibiotics and to prepare the bacterial inoculums.

The MICs of the untreated antibiotic samples and heat-treated antibiotic samples (after exposure to heat at 121°C for 15 minutes in an autoclave) against the tested bacteria were determined. If the MIC of the heat-treated antibiotic sample had increased by more than twofold the MIC of the untreated antibiotic sample, the MIC of such antibiotic was additionally determined after heating the antibiotic sample at 100°C for 30 minutes. Each MIC determination of each antibiotic sample against each bacterium for each condition was performed in duplicate. A heat-stable antibiotic was defined as one for which the MIC of the heat-treated antibiotic was not more than twofold the MIC of the untreated antibiotic[6]. A heat-labile antibiotic was defined as one for which the MIC of the heat-treated antibiotic was more than twofold the MIC of the untreated antibiotic[6].

Results

The MICs of the heat-labile antibiotics against the tested bacteria are at Table 1. Bacitracin and neomycin sulphate were heat-labile at 100°C and 121°C, while amoxicillin and amoxicillin/clavulanate seemed to be heat-stable at 100°C but heat-labile at 121°C. The MICs of the heat-stable antibiotics against the tested bacteria are at Table 2. The MICs of the heated tetracycline samples at 121°C were two- to four-fold the MICs of the untreated tetracycline samples. Enrofloxacin, gentamicin, kanamycin, tiamulin, tilmicosin, tylvalosin and colistin were quite stable to heat at 121°C.

Discussion

Amoxicillin, amoxicillin/clavulanate, tetracyclines, enrofloxacin, gentamicin, kanamycin, tiamulin, tilmicosin, bacitracin/neomycin and colistin were selected for this study because they are commonly used for the prevention and treatment of infections in food animals and agriculture in Thailand. The antibiotic samples were processed by heating them at 100°C for

Table 1. MICs of the heat-labile antibiotics against the tested bacteria

Antibiotic	*S. aureus* ATCC 29213				*E. coli* ATCC 25922				*B. subtilis* ATCC 6633			
	MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 100°C (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)		MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 100°C (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)		MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 100°C (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)	
Bacitracin 75 IU/ Neomycin sulphate 2.5 mg	64	64	1024	1024	1024	1024	512	512	2048	2048	2048	2048	32	32	512	512	256	256
Amoxicillin 50%	0.25	0.25	0.5	0.5	4	4	8	8	16	16	128	128	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	1	1
Amoxicillin 50 mg/ Clavulanate 12.5 mg	0.25	0.25	0.5	0.5	2	2	8	8	8	16	64	64	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	4	8
Amoxicillin 100 mg/ Clavulanate 25 mg	0.25	0.25	0.5	0.5	8	8	8	8	8	8	64	64	≤ 0.12	≤ 0.12	≤ 0.12	≤ 0.12	4	8

Table 2. MICs of the heat-stable antibiotics against the tested bacteria

Antibiotic	*S. aureus* ATCC 29213				*E. coli* ATCC 25922				*B. subtilis* ATCC 6633			
	MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)		MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)		MIC of untreated antibiotic sample (mg/L)		MIC of heat-treated antibiotic sample at 121°C (mg/L)	
Oxytetracycline 50 mg/mL	0.5	0.5	1	1	1	1	2	2	0.25	0.12	0.25	0.5
Chlortetracycline 20%	2	2	8	8	8	8	16	16	0.5	0.5	2	2
Doxycycline 10%	0.06	0.06	0.25	0.25	0.5	0.5	1	1	≤0.03	≤0.03	0.06	0.06
Enrofloxacin 50%	0.12	0.12	0.12	0.12	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	0.06
Enrofloxacin 20%	0.25	0.12	0.12	0.12	≤0.03	≤0.03	≤0.03	≤0.03	0.06	0.06	≤0.03	≤0.03
Enrofloxacin 50 mg/mL	0.25	0.25	0.25	0.25	≤0.03	≤0.03	≤0.03	≤0.03	0.06	0.12	0.12	0.12
Gentamicin 40 mg/mL	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0.25	0.25	0.25	0.12	0.12
Kanamycin 25 g/100 mL	2	4	2	2	2	4	2	1	1	0.5	0.5	0.5
Tiamulin 10%	1	1	1	1	>16	>16	>16	>16	>16	>16	>16	>16
Tiamulin 25%	1	1	1	1	>16	>16	>16	>16	>16	>16	>16	>16
Tilmicosin 20%	2	2	4	4	>16	>16	>16	>16	2	2	1	1
Tylvalosin 62.5%	4	4	4	4	>16	>16	>16	>16	1	1	1	1
Colistin 40%	>16	>16	>16	>16	2	2	2	2	16	16	16	16

30 minutes and 121°C for 15 minutes in order imitate the temperatures used for boiling and frying food, and for the autoclaving of biological samples. Instead of measuring the amount of leftover antibiotics after heating, a microbiological test was chosen to determine the functional activity of the antibiotics. The tested bacteria, namely, *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *B. subtilis* ATCC 6633, were selected for use in the study because they are usually susceptible to various kinds of antibiotics, allowing the antimicrobial activity of the tested antibiotics to be detected.

Our study results confirmed the findings of a previous study on the heat stability of 62 antibiotics used in humans that most beta-lactams, especially ampicillin, amoxicillin, and beta-lactam with beta-lactamase inhibitor, were heat-labile agents, whereas aminoglycosides and fluoroquinolones were heat-stable agents[6]. Our study results revealed that several antibiotics that are usually used in food animals, i.e., enrofloxacin, tiamulin, tilmicosin, tylvalosin and colistin, were also heat-stable agents. Tetracyclines were observed to be rather stable to heat in our study, whereas the previous study reported that they were heat-labile. Although cooking food with heat at 100°C and 121°C can kill AMR bacteria, the heat is unable to inactivate many antibiotics commonly used in food animals and agriculture. Therefore, heat-cooked foods containing heat-stable antibiotics still pose a risk of inducing AMR in humans who consume such foods. The autoclaving of heat-stable antibiotics before their disposal would not inactivate their antimicrobial activity, which means the autoclaved heat-stable antibiotics could still induce AMR in the environment. It should be noted that heat-labile antibiotics are also important in terms of food safety since much food containing heat-labile antibiotics is consumed as fresh products. An example is orange juice made from oranges harvested from orange trees injected with antibiotics (usually ampicillin, amoxicillin and tetracycline) to prevent or treat greening disease.

Farmers should be aware of the harmful effects of antibiotic residues in the products from their farms, especially in the case of heat-stable antibiotics, and they should avoid using unnecessary antibiotics, especially for growth promotion and infection prevention. If antibiotics are really needed for food animals and agriculture, the products should be harvested after a sufficient withdrawal period from using the antibiotics. The Ministry of Agriculture and Cooperatives has been taking steps to limit the use of antibiotics in food animals over recent years, including issuing a regulation that antibiotics were not permitted to be used as a growth promoter in food animals from August 2015. Moreover, several regulations regarding the use of colistin in food animals have been in force since February 2017. Colistin is no longer permitted to be used to prevent infection in food animals. Colistin has to be prescribed by a responsible veterinarian for the short-term treatment of infections in food animals only; the veterinarian must report the amount of colistin used to the provincial livestock office.

Our study had several limitations. The data on the heat stability of antibiotics after heating at 100°C for longer than 30 minutes and 121°C for longer than 15 minutes were not available from our study. The antimicrobial activity of the heat-treated antibiotics was performed in vitro with the antibiotics before consumption by humans. Therefore, the antimicrobial activities of the metabolic products of the tested antibiotics were unknown. The stability of the antibiotics was determined by their antimicrobial activity against *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and *B. subtilis* ATCC 6633. Consequently, the heat-labile antibiotics after heating may be capable of inducing AMR in bacteria in humans that are very susceptible to minute activities of such heat-labile antibiotics.

Conclusion

Many antibiotics commonly used in food animals and agriculture in Thailand are heat-stable. Therefore, ingestion of heat-cooked foods containing heat-stable antibiotic residues could still present a threat for the emergence of antibiotic-resistant bacteria in humans.

What is already known on this topic?

Some foods derived from animal products and agriculture in Thailand contain antibiotic residues. No data on the heat stability of antibiotics commonly used in food animals and agriculture in Thailand are available.

What this study adds?

Many antibiotics commonly used in food animals and agriculture in Thailand are heat-stable. Therefore, ingestion of heat-cooked foods containing heat-stable antibiotic residues could still pose a threat of the emergence of antibiotic-resistant bacteria in humans.

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Potential conflicts of interest

The authors declare no conflict of interest.

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