RESEARCH ARTICLE
Open Access

Mandarin farmers' knowledge, attitudes, and practices related to antibiotic use in mandarin orchards and the presence of antibiotic-resistant bacteria in mandarin orchard workers in Fang district, Chiang Mai province, Thailand

Mewika Hamtook[1], Parichat Ongartborirak[1], Aksara Thongprachum[1], Usanee Wattananandkul[2], and Warangkana Naksen[1]

1. Faculty of Public Health, Chiang Mai University, Chiang Mai 50200, Thailand; 2. Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Muang District, Chiang Mai 50200, Thailand.
Corresponding author: Warangkana Naksen, e-mail: warangkana.n@cmu.ac.th
Co-authors: MH: mewika_h@cmu.ac.th, PO: parichat.ong@cmu.ac.th, AT: aksara.t@cmu.ac.th, UW: usanee.anukool@cmu.ac.th
Received: 14-05-2023, **Accepted:** 22-08-2023, **Published online:** 26-09-2023

doi: www.doi.org/10.14202/IJOH.2023.95-105 **How to cite this article:** Hamtook M, Ongartborirak P, Thongprachum A, Wattananandkul U, and Naksen W (2023) Mandarin farmers' knowledge, attitudes, and practices related to antibiotic use in mandarin orchards and the presence of antibiotic-resistant bacteria in mandarin orchard workers in Fang district, Chiang Mai province, Thailand, *Int. J. One Health*, 9(2): 95–105.

Abstract

Background and Aim: Inappropriate antibiotic use in the agricultural sector is a crucial driver of antibiotic resistance which is a global public health concern. Although there are many studies on antibiotic use in livestock production, studies on antibiotic use in crop production are relatively scarce. This study aimed to determine farmers' knowledge, attitudes, and practices (KAP) toward antibiotic use in Mandarin orchard and investigate their associations with the antibiotic susceptibility patterns among Mandarin orchard farmers in Fang District, Chiang Mai Province, Thailand.

Materials and Methods: This cross-sectional study was conducted in Fang District between November and December 2021. The participants in this study were 151 farmers (farm owners and farm workers) in ten Mandarin orchards. All participants completed face-to-face interviews using a structured questionnaire. Nasal swabs were collected from 100 participants. The disk diffusion and staphylococcal chromosome cassette *mec* typing methods were used to test the antibiotic susceptibility of the isolates.

Results: All farmers involved in the activities related to antibiotic exposure, 39.7% prepared or mixed antibiotics, and 82.8% injected antibiotics into Mandarin trees. Overall, farmers in this study had moderate levels of knowledge and attitudes and good antibiotic use practices. There was a significant association between attitudes and practices ($r = 0.312$, $p < 0.001$). In the analysis of drug resistance of the bacterial isolates, *Staphylococcus aureus* was detected in 16 of 100 nasal swab samples, with resistance to penicillin (16/16), methicillin (cefoxitin) (1/16), and tetracycline (6/16).

Conclusion: This is the first report on farmers' and farm workers' KAP and antibiotic use in crop production, specifically Mandarin production. The findings of this research will help in developing plans and strategies to encourage the appropriate use of antibiotics and prevent antibiotic resistance or microbial resistance in plant production.

Keywords: antibiotic use, attitudes, farmers, knowledge, mandarin orchard, practices.

Introduction

Antibiotic drugs play a very important role in medicine and public health in the prevention and treatment of bacterial infections. However, the unreasonable use of antibiotics causes bacteria to adapt and develop to become resistant to antibiotics [1]. According to the World Health Organization, the current trend of antibiotic resistance continues to increase. If preventive measures cannot be found, it may lead to the post-antibiotic era, where common infections could become untreatable [2]. This seriously threatens health security and causes economic and social losses.

According to the report of the Interagency Coordination Group on Antibiotic Resistance, around 700,000 people die each year from antibiotic-resistant infections globally. By 2050, deaths are expected to reach 10 million, with Asia and Africa having the highest death tolls (4.7 and 4.2 million, respectively). The potential economic impact is expected to be up to 3500 million Baht or 100 million USD [3]. In Thailand, the data show that there are approximately 38,000 deaths from drug-resistant bacteria per year, representing a total economic loss of 42 billion Baht or 1.2 billion USD [4].

Besides using antibiotics to treat disease in humans, antibiotics have been commonly used in agricultural and livestock production sectors for controlling and preventing the spread of infection or disease in many regions, including Thailand [5, 6]. In

Thailand, in Mandarin (*Citrus reticulata* Blanco) production, antibiotics such as ampicillin, amoxicillin, and tetracycline have been used to manage citrus diseases such as greening and Canker diseases through trunk injection [5]. Based on the literature review and the pilot interview, the orchard owners and workers were instructed to apply antibiotics to Mandarin trees appropriately and safely by the academic sector. After that, they applied antibiotics based on their experience and did not follow the appropriate instructions [5].

Fang District, Chiang Mai Province, is a major area of Mandarin production in Thailand. As reported in previous studies, antibiotics are used in most orchards in this area for crop protection [5, 7]. One research group found that antibiotics, such as ampicillin, contaminated Mandarin products and the environment, such as water samples, collected from the Fang River Basin located in the area (unpublished). Moreover, there is evidence of antibiotic-resistant bacteria found in the environment (soil, water, and Mandarin surface) and in samples (nasal and hand) from Mandarin farmers [8]. Mandarin farmers and people living in nearby areas might be exposed to antibiotics and infected by antibiotic-resistant bacteria. They should be informed of the risks of antibiotic contamination and educated about the proper use of antibiotics to increase awareness of antibiotic or antimicrobial resistance (AMR).

Many studies in various countries, including developing countries like Thailand, have surveyed livestock farmers' knowledge, attitudes, and practices (KAP) regarding antibiotic use [9–16]. Similar surveys on antibiotic use in plant production and antibiotic susceptibility patterns are scarce.

Therefore, this study aimed to determine Mandarin farmers' KAP related to antibiotic use and the association of KAP with antibiotic susceptibility patterns among Mandarin orchard workers in Fang District, Chiang Mai Province.

Materials and Methods

Ethical approval and informed consent

This research involving human participants was reviewed and approved by the Ethics Committee of Faculty of Public Health, Chiang Mai University (Approval document no. is ET016/2564). All samples and data were collected after obtaining written informed consent from the participants. A trained medical technician collected the nasal swabs.

Study period and location

This research was conducted between November and December 2021. All data and nasal swab samples were collected from farmers operating in ten Mandarin orchards in Fang District, Chiang Mai Province, upper northern Thailand, located between latitude 19° 55′ 8″ N, and longitude 99° 12′ 49″ E, which is an important area for Mandarin cultivation (Figure-1). Based on data from the Fang District Agriculture Office on citrus growers in Fang District, Chiang Mai Province, there are 758 Mandarin orchards and 2680 Mandarin farmers.

Study design

This cross-sectional study used a quantitative method to study KAP on antibiotic use and drug-resistant bacteria among Mandarin orchard farmers in Fang District, Chiang Mai Province. The data on KAP regarding antibiotic use were collected in individual face-to-face interviews using a structured questionnaire. Nasal swabs were collected, and data on antibiotic-resistant bacteria were screened from the collected biological samples.

Participants

The participants in this study were 151 farmers, including Mandarin orchard owners and workers, in ten Mandarin orchards in Fang District, Chiang Mai Province. The number of participants was calculated based on a sample size calculation using correlation testing. The samples were conveniently selected from ten orchards willing to participate in the study. Multistage sampling was conducted. First, ten Mandarin orchards of various sizes were randomly sampled. Then, 10–15 farmers who met the following inclusion criteria were recruited: farmers who owned a Mandarin orchard or workers in a Mandarin orchard in which antibiotics were used for at least 1 year, aged between 18 and 70 years, able to communicate in Thai, and willing to participate in the study.

Knowledge, attitudes, and practices data collection

A structured questionnaire was used to assess the participants' KAP regarding antibiotic use in Mandarin orchards based on a literature review and preliminary field survey. The structured questionnaires were administered to the participants through face-to-face interview. The questionnaire consisted of 4 parts: demographics and information about work-related practices and antibiotic use in the orchard (checklists), knowledge (20 questions), attitudes (20 questions), and practices (11 questions).

The knowledge score was derived from the actual points from every correct answer (true or false) to 20 questions. Attitude was scored on a five-point Likert scale ranging from 1 to 5. For positively worded questions: 1 = strongly disagree, 2 = disagree, 3 = neither agree nor disagree/do not know, 4 = agree, and 5 = strongly agree; for negatively worded questions, reverse scoring was used. Likewise, practice was scored on a 5-point Likert scale ranging from 1 to 5. For positively worded questions: 1 = never, 2 = seldom, 3 = sometimes, 4 = often, and 5 = always; for negatively worded questions; reverse scoring was used. The knowledge score was categorized into three levels using Bloom's cutoff point as follows: low (<60%), moderate (60–80%), and high (more than 80%) levels. The attitude and practice scores were interval-equally categorized into three levels which were high, medium, and low.

The questionnaire was reviewed and evaluated for content validity by three experts in antibiotics and

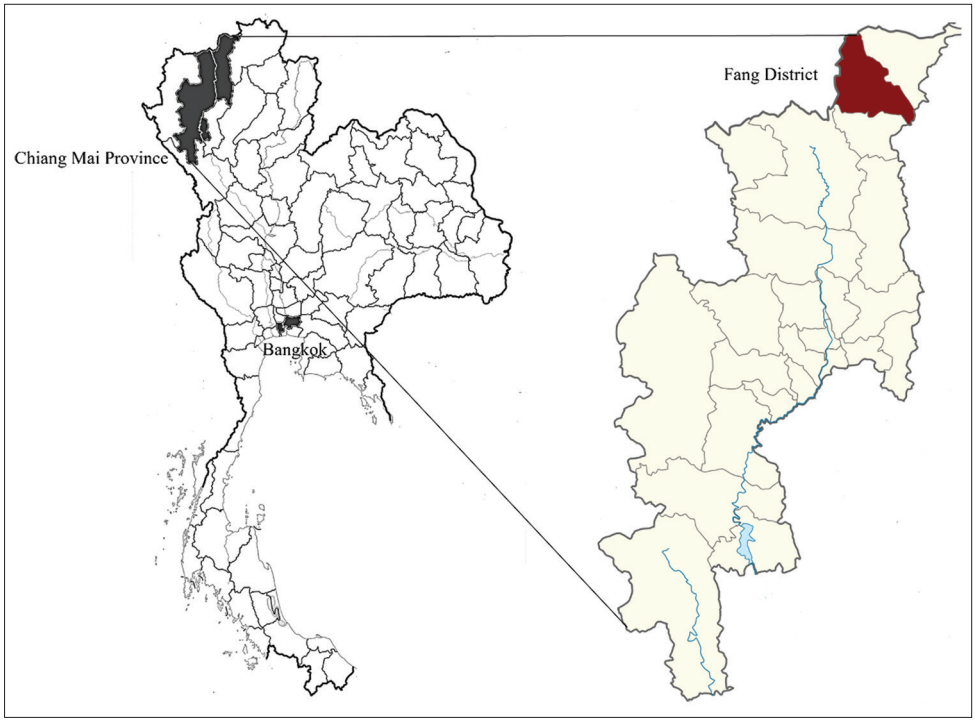

Figure-1: The location of study area.

Mandarin cultivations. The KAP questionnaire was pretested among 30 Mandarin farmers from other Mandarin orchards and tested for reliability. The Kuder-Richardson Formula 20 for knowledge was 0.84, while Cronbach's alpha for attitude and practice was 0.75 and 0.72, respectively.

Collection of nasal swabs

Nasal swabs were collected from 100 participants for screening for antibiotic-resistant bacteria. The nasal screening samples were obtained by rotating sterile swabs (1-mL Liquid Amies Regular Flocked Swab, eSwab®, COPAN, Italy) over the nasal vestibule. The swab procedure followed the Standard Guideline for Microbiological Laboratory of the Department of Medical Sciences, Ministry of Public Health [17]. The nasal swab samples were collected using new and clean equipment and containers for sampling. Once the samples were collected, the temperature was maintained by freezing them in a Styrofoam box throughout the time of transportation to the laboratory. All samples were stored at −20°C in a freezer until the analysis.

Antibiotic susceptibility testing and Staphylococcal chromosome cassette mec (SCCmec) typing for methicillin-resistant *Staphylococcus aureus* (MRSA)

Bacterial isolation and identification, disk diffusion antibiotic susceptibility testing, and SCC*mec* typing were performed as described in a previous study [18]. Out of the total 100 nasal swab samples tested in this study, the prevalence of *S. aureus* was found to be 16. Twenty-one *S. aureus* isolates were tested for antibiotic susceptibility. The following antibiotics (Sensi-Discs, Becton, Dickinson and Company, Loveton, USA) and disk potencies were used: penicillin (10 Units), erythromycin (15 μg), clindamycin (2 μg), trimethoprim-sulfamethoxazole (25 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), gentamicin (10 μg), rifampin (5 μg), tetracycline (30 μg), cephazolin (30 μg), fosfomycin (50 μg), and linezolid (30 μg). The SCC*mec* types of MRSA isolates were characterized by the multiplex polymerase chain reaction. All laboratory analyses were performed at the laboratory of the Faculty of Associated Medical Sciences, Chiang Mai University.

Statistical analysis

The collected data were processed and analyzed using the statistical package for the social sciences software, version 25.0 (IBM Corp., Armonk, NY, USA). Demographics and the information about and work-related practices related to antibiotics and their use in the orchard were described as frequency, percentage, mean, median, standard deviation (SD), and interquartile range (IQR). Knowledge, attitudes, and practices scores were reported as mean, median, SD, and IQR. The scores were interpreted as a high, moderate, or low level of knowledge. The associations between KAP and demographic data were tested. Mode imputation (or mode substitution) was used to replace missing values of categorical variables. An independent t-test and one-way analysis of variance (ANOVA) were used to test the mean difference between or among two or more categorical variables. Knowledge, attitudes, and practices scores were normally distributed as revealed by the absolute values of skewness (<1). Pearson's correlations were used to investigate the associations between two continuous variables. $p < 0.05$ was considered statistically significant.

Results

Demographics and work-related practices related to antibiotics and their use in Mandarin orchards

Of the 151 Mandarin orchard farmers interviewed in this study, 52.3% were female. The average age of the participants was 34.9 ± 13.0 years old. Most farmers (76.2%) were not educated. Most orchards were large (as determined by the number of Mandarin trees). The majority of the farmers worked approximately 20 h per week. The most frequently used method of antibiotic application in the Mandarin orchards was injecting the antibiotic into the tree trunk using the vacuum (87.4%) followed by injecting the antibiotic into the trunk using the syringe (37.7%) (Table-1).

All farmers involved in activities related to antibiotic exposure, 60.3% prepared or mixed antibiotics, and 82.8% injected antibiotics into the trunk. Almost 60% of farm workers applied antibiotics on the instructions of their employers and were unaware of which antibiotic was used. Antibiotics were applied to the trees 2–3 times per year, regardless of the season. In most cases, tree size determined decisions related to antibiotic dosages. While applying the antibiotics, most of the participants in this study said that they wore a surgical mask, a hat, a long-sleeved shirt, long pants, and boots (>80%). Cloth and nitrile gloves, goggles, and a full-body gown were worn less often.

Knowledge regarding antibiotic use in Mandarin orchards

Overall, most farmers (58.9%) had a moderate level of knowledge regarding antibiotic use in Mandarin orchard, with the mean score of knowledge at 7.86 ± 3.53 (Tables-1 and 2). Considering the item-based questions, more than half of farmers had no knowledge of proper antibiotic use, management of used containers, antibiotics registration in plant cultivation, and antibiotic resistance or AMR. On the other hand, farmers had good knowledge about the antibiotic storage and withdrawal period before harvesting.

Attitudes regarding antibiotic use in Mandarin orchards

Approximately 80% of farmers had a moderate level of attitudes regarding antibiotic use in Mandarin orchard with a mean score of 56.2 ± 9.74 (Tables-1 and 3). Most farmers (74.2%) strongly agreed that antibiotics are needed for Mandarin cultivation and 70% agreed that applying antibiotics is the most effective way of treating diseases of Mandarin trees.

Practices regarding antibiotic use in Mandarin orchards

Tables-1 and 4 provide information on antibiotic use practices in the Mandarin orchards. Considering antibiotic application practices, half of the farmers had good practices, with a mean score of 39.8 ± 6.43. Most farmers (>80%) wore clothes that covered their bodies, such as long-sleeved shirts, long pants, and boots, but fewer wore personal protective equipment (PPE) that covered their faces, such as masks and goggles or glasses, when applying antibiotics in the orchards. Almost 70% of those surveyed treated the trees in the Mandarin orchards with antibiotics, despite the absence of signs or symptoms of diseases.

Prevalence of *S. aureus*

In the analysis of drug resistance from 100 nasal swab samples from Mandarin orchard farmers in Fang District, 282 colonies of bacteria were isolated. Antibiotic resistance was detected in 21 *S. aureus* colonies isolated from 16 nasal swab samples, which tended to be resistant to penicillin (16/16), methicillin (cefoxitin) (1/16), and tetracycline (6/16) (Table-5). All 21 isolates were resistant to penicillin, and a single isolate was identified as MRSA by cefoxitin disk diffusion test and PCR detection of the *mecA* gene. However, the SCC*mec* was untypeable using multiplex PCR method, as the *mec* class could not be identified, although the *ccrA2-B2* was detected.

Association of KAP with antibiotic use in Mandarin orchards and AMR

Pearson's correlation test revealed a significant association between attitude and practice scores ($r = 0.312$, $p < 0.001$) but no associations of knowledge with attitude ($r = 0.144$, $p = 0.078$) or knowledge with practices ($r = 0.087$, $p = 0.286$). Sex (female and male), working status (orchard owner and farm workers), education level (uneducated, primary school, high school, and bachelor's degree and higher), and detected antibiotic-resistant bacteria (positive and negative) were not related to KAP scores according to independence t-test and one-way ANOVA test. In addition, age and working experience in Mandarin orchards were not correlated with KAP scores as revealed by Pearson's correlation test.

Discussion

In Thailand, antibiotics are widely used in the agricultural sector, involving food animal and crop production. In livestock farming in Thailand, antibiotic usage has been regulated by the Department of Livestock Development since 2003. Thailand has banned antibiotics as growth promoters since 2015 and classified veterinary antibiotics as dangerous drugs that could only be obtained through licensed pharmacists or veterinarians at authorized pharmacies [6]. At present, there are no legal guidelines governing the use of antibiotics in commercial fruit production in Thailand, including Mandarin production [4, 5]. Therefore, antibiotics are applied by farmers in Mandarin orchards in Thailand in the absence of any established guidelines or knowledge base.

According to our current literature review, there is no report on KAP regarding antibiotic use in crop production. This study surveyed KAP toward antibiotic use in Mandarin orchards and investigated their associations with positive antibiotic resistance among Mandarin

Table-1: Demographics and self-reported information about working and antibiotic use in Mandarin orchard (n = 151).

Variables	Frequency	Percentage
Sex		
Female	79	52.3
Male	72	47.7
Age: year (mean ± SD)	34.3 ± 12.8	
Working experience in Mandarin orchard: year (mean ± SD)	6.68 ± 7.13	
Working status		
Orchard's owner	34	22.5
Worker	117	77.5
Education level		
Uneducated	115	76.2
Primary school	18	11.9
High school	12	7.9
Bachelor's degree and higher	6	4.0
Responsible for preparing or mixing antibiotics		
Yes	91	60.3
No	60	39.7
Responsible for injecting antibiotics into the trunk		
Yes	125	82.8
No	26	17.2
Reasons of antibiotic use (can select more than 1 answer)		
Treating stem and root rot diseases	29	19.2
Treating greening disease	41	27.2
Treating scab disease	3	2.0
Treating black spot disease	5	3.3
Following orchard owner's order	89	58.9
Type of antibiotic used use (can select more than 1 answer)		
Tetracycline	3	2.0
Penicillin	1	0.7
Amoxicillin	7	4.6
Don't know	86	57.0
Method of antibiotic applied to Mandarin tree (can select more than 1 answer)		
Pouring on the soil around the tree	1	0.7
Spraying on the leaves	1	0.7
Injecting using the syringe into the trunk	57	37.7
Injecting using the vacuum bottle into the trunk	132	87.4
Timing of antibiotic use		
Winter	3	2.0
Summer	3	2.0
Rainy	3	2.0
Season-independent	140	92.7
Missing	2	1.3
PPE wearing		
Hat	135	89.4
Surgical/cloth mask	121	80.1
Goggle/glasses	16	10.6
Cloth gloves	61	40.4
Nitrile gloves	65	43.0
Coverall gown	10	6.6
Long-sleeved shirt	147	97.4
Long pants	148	98.0
Boots	138	91.4
Sources of recommendations/advice on antibiotic use in the orchard (can select more than 1 answer)		
Neighbors/friends	34	22.5
Employers/orchard's owners	108	71.5
Agrochemical retail stores	7	4.6
Drug stores	3	2.0
Academic expert	6	4.0
Related governmental office	0	0.0
Self-learning	10	6.6
Sources of antibiotic used in the orchard		
Neighbors/friends	5	3.3
Employers/orchard's owners	103	68.2
Agrochemical retail stores	24	15.9
Drug stores	28	18.5
Academic expert	1	0.7

(Contd...)

Table-1: (*Continued*).

Variables	Frequency	Percentage
Related governmental office	0	0.0
Knowledge score (mean ± SD)	7.86 ± 3.53	(Low)
Low	53	35.1
Medium	89	58.9
High	9	6.0
Attitude score (mean ± SD)	56.2 ± 9.74	(Low)
Low	22	14.6
Medium	124	82.1
High	5	3.3
Practice score: antibiotic applying practices (mean ± SD)	39.8 ± 6.43	(Moderate)
Low	4	2.6
Medium	67	44.4
High	80	53.0
Detected antibiotic-resistant bacteria (n = 100)		
Positive	16	10.6
Negative	84	55.6

PPE=Personal protective equipment

Table-2: The item-based questions about knowledge regarding antibiotic use in Mandarin orchard (n = 151).

Statements (correct answer)	Number of correct answer (%)
1. Antibiotics used in Mandarin orchard are similar to antibiotics used in humans. (True)	86 (57.0)
2. Antibiotics can treat fungal infections in Mandarin trees. (False)	16 (10.6)
3. Antibiotics can treat bacterial infections in Mandarin trees. (True)	64 (42.4)
4. Antibiotics can treat Greening disease in Mandarin trees. (True)	64 (42.4)
5. Antibiotics can directly enhance the growth of Mandarin trees. (False)	28 (18.5)
6. The amount and type of antibiotics used with Mandarin trees depended on disease type and tree size. (True)	119 (78.8)
7. Using antibiotics in Mandarin orchards should be under expert's supervision. (True)	44 (29.1)
8. Antibiotics should be kept in a proper storage area according to the manufacturer's recommendation. (True)	114 (75.5)
9. Taking the same antibiotic for an extended period has no adverse effect on Mandarin tree. (False)	30 (19.9)
10. Antibiotics should be applied to Mandarin trees regularly, without consideration of occurring symptoms or diseases. (False)	17 (11.3)
11. Using antibiotics in Mandarin orchard leads to antibiotic contamination in the product. (True)	49 (32.5)
12. Using antibiotics in Mandarin orchard can indirectly increase the probability of exposure via eating contaminated fruits. (True)	42 (27.8)
13. The main cause of antibiotic resistance is antibiotic overuse. (True)	58 (38.4)
14. Inappropriate antibiotic use is recognized as the primary driver of antibiotic resistance. (True)	50 (33.1)
15. Antibiotic contamination in the environment can cause antibiotic resistance in humans and animals. (True)	37 (24.5)
16. Reasonable antibiotic use and reduction of use of antibiotics can reduce the risk of antibiotic resistance. (True)	41 (27.2)
17. Antibiotics can be applied to Mandarin trees during harvesting. (False)	81 (53.6)
18. There are no registered antibiotics to be used in plants in Thailand. (True)	38 (25.2)
19. Antibiotics used in the orchard should be kept in a separate area away from other agricultural chemicals. (True)	123 (81.5)
20. Antibiotics used in the orchard should be kept in a safe and proper place away from people and children, but not pets. (False)	35 (23.2)

orchard farmers in Fang District, Chiang Mai Province, which is an intensive Mandarin-cultivating area in Thailand. The majority of the farmers in this study had moderate levels of knowledge (58.9%) and attitudes (82.1%) and high level of practice (53%) regarding antibiotic use in Mandarin orchards. In a previous study in a similar area in Thailand, farmers involved in livestock production had low levels of knowledge, neutral attitudes, and poor practices related to antimicrobial usage [19].

The majority of the participants in this study believed that antibiotics could treat fungal infections and promote the growth of Mandarin trees. This finding is in contrast to a previous study on farmers on layer and pig farms, in which a high proportion of participants knew that antibiotics cannot be used to treat fungal infections [19]. The discord between the studies may be the result of different experiences and varying knowledge levels among different production

Table-3: The item-based questions about attitudes regarding antibiotic use in Mandarin orchard (n = 151).

Questions	Frequency (%)				
	Strongly agree	Agree	Neither agree nor disagree/ do not know	Disagree	Strongly disagree
1. Do you think that antibiotics are needed for Mandarin cultivation? (negative)	112 (74.2)	19 (12.6)	9 (6.0)	2 (1.3)	9 (6.0)
2. Do you think that applying antibiotics is the most effective way of disease treatment for Mandarin trees? (negative)	106 (70.2)	26 (17.2)	8 (5.3)	3 (2.0)	8 (5.4)
3. Do you think that using antibiotics in Mandarin orchards for a long period can promote antibiotic resistance? (positive)	47 (31.1)	24 (15.9)	38 (25.2)	10 (6.6)	32 (21.2)
4. Do you think that using antibiotics in Mandarin orchards should be under expert's supervision? (positive)	45 (29.8)	14 (9.3)	29 (19.2)	35 (23.2)	28 (18.5)
5. Do you think that antibiotics used in Mandarin orchard are not harmful to human's health because they are the same drugs normally used in human? (negative)	14 (9.3)	14 (9.3)	37 (24.5)	27 (17.9)	59 (39.1)
6. Do you think that farmers who apply antibiotic in Mandarin orchard do not need to wear personal protective equipment because antibiotics are the same drugs normally used in human? (negative)	48 (31.8)	24 (15.9)	21 (13.9)	24 (15.9)	34 (22.5)
7. Do you think antibiotics used in Mandarin orchard can contaminate the environment? (positive)	30 (19.9)	40 (26.5)	31 (20.5)	18 (11.9)	32 (21.2)
8. Do you think that antibiotics used in Mandarin orchard can legally be bought in general drug stores? (negative)	68 (45.0)	30 (19.9)	14 (9.3)	11 (7.3)	28 (18.5)
9. Do you think that increasing amount of antibiotics used in Mandarin orchard can increase crop yields? (negative)	82 (54.3)	25 (16.6)	15 (9.9)	13 (8.6)	16 (10.6)
10. Do you think that using stronger antibiotics can increase crop yields in Mandarin orchard? (negative)	63 (41.7)	25 (16.6)	15 (9.9)	16 (10.6)	32 (21.2)
11. Do you think that reasonable antibiotic use in Mandarin orchard can reduce risk of antibiotic resistance? (positive)	40 (26.5)	29 (19.2)	50 (33.1)	18 (11.9)	14 (9.3)
12. Do you think that appropriate management of waste container of antibiotic used in Mandarin orchard can reduce the contamination in the environment? (positive)	47 (31.4)	34 (22.5)	32 (21.2)	17 (11.3)	21 (13.9)
13. Do you think that antibiotics can be used in Mandarin orchard without any legal guilt? (negative)	71 (47.0)	22 (14.6)	29 (19.2)	12 (7.9)	17 (11.3)
14. Do you think that intensive regulatory control and law enforcement on antibiotic use in Mandarin orchard can solve the antibiotic resistance problem? (positive)	27 (17.9)	14 (9.3)	63 (41.7)	19 (12.6)	28 (18.5)
15. Do you think that antibiotics used in the orchard should be kept in a safe and proper place away from people and children? (positive)	93 (61.6)	31 (20.5)	9 (6.0)	8 (5.3)	10 (6.6)
16. Do you think that farmers who are frequently exposed to antibiotics used in Mandarin orchard can gain benefits to their health? (negative)	30 (19.9)	22 (14.6)	23 (15.2)	31 (20.5)	45 (29.8)
17. Do you think that monitoring the antibiotic residues in Mandarin products before harvesting is necessary? (positive)	31 (20.5)	54 (35.8)	30 (19.9)	16 (9.9)	26 (7.2)
18. Do you think that monitoring the antibiotic residues in Mandarin products in the market before selling is necessary? (positive)	31 (21.2)	56 (35.0)	30 (20.5)	13 (8.6)	23 (15.2)
19. Do you think that antibiotics applied to Mandarin trees through trunk injection cannot contaminate Mandarin crops? (positive)	25 (16.6)	12 (7.9)	24 (15.9)	48 (31.8)	42 (27.8)
20. Do you think that type and amount of antibiotics used in Mandarin tree can be determined by farmers' decision. (negative)	32 (21.2)	50 (33.1)	24 (15.9)	20 (13.2)	25 (16.6)

sectors. In this study, most farmers believed that antibiotics should be applied to Mandarin trees regularly, without considering symptoms or diseases. This application practices, in which they applied antibiotics to Mandarin trees throughout the year. Such practice can negatively affect the health of humans, animals, and the environment [5]. Moreover, the farmers had inadequate knowledge about the overuse of antibiotics and antibiotic resistance, which is in accordance with the findings of other studies among livestock farmers [19, 20]. Knowledge and understanding of antibiotic resistance can be enhanced through effective communication, education, and training [9].

In our findings, many farmers had positive attitudes about the use of antibiotics in Mandarin production. They believed that antibiotics are needed for

Table-4: The item-based statements about practices regarding antibiotic use in Mandarin orchard.

Statements	Frequency (%)				
	Never	Seldom	Sometimes	Often	Always
Antibiotic applying practices (n = 151)					
1. You apply antibiotics in the Mandarin orchard, although there are no signs or symptoms of disease. (negative)	14 (9.3)	3 (2.0)	12 (7.9)	18 (11.9)	104 (68.9)
2. You wear clothes that cover your body, such as a long-sleeved shirt and long pants, when applying antibiotics in the orchard. (positive)	2 (1.3)	2 (1.3)	1 (0.7)	15 (9.9)	131 (86.8)
3. You wear PPE that covers your face, such as a mask and goggles or glasses, when applying antibiotics in the orchard. (positive)	38 (25.2)	18 (11.9)	25 (16.6)	16 (10.6)	54 (35.8)
4. You wear PPE that protects your hands from chemicals, such as nitrile gloves, when applying antibiotics in the orchard. (positive)	59 (39.1)	7 (4.6)	5 (3.3)	17 (11.3)	63 (41.7)
5. You wear PPE that covers your feet, such as boots, when applying antibiotics in the orchard. (positive)	5 (3.3)	1 (0.7)	2 (1.3)	14 (9.3)	129 (85.4)
6. You wash your body immediately after applying antibiotics in the Mandarin orchards. (positive)	16 (10.6)	2 (1.3)	11 (7.3)	17 (11.3)	105 (69.5)
7. You stop applying antibiotics in the Mandarin orchard during the harvesting period. (positive)	19 (126)	3 (2.0)	9 (6.0)	21 (13.9)	99 (65.6)
8. You keep antibiotics used in the orchard in a safe and proper place away from people and children. (positive)	8 (5.3)	5 (3.3)	5 (3.3)	26 (17.2)	107 (70.9)
9. You dispose of used antibiotics and their empty containers in the household trash. (negative)	53 (35.1)	15 (9.9)	26 (17.2)	16 (10.6)	41 (27.2)
10. You reuse used antibiotic containers without rinsing or cleaning them. (negative)	75 (49.7)	10 (6.6)	5 (3.3)	13 (8.6)	48 (31.8)
11. You smoke or drink or eat while processing antibiotics in the orchard. (negative)	48 (31.8)	10 (6.6)	20 (13.2)	21 (13.9)	52 (34.4)
Preparing and mixing practices (only farmers who has a responsibility in preparing and mixing antibiotics) (n = 91)					
1. You use the amount of antibiotics with the Mandarin trees according to the expert's recommendation. (positive)	23 (41.1)	0 (0.0)	10 (17.9)	4 (7.1)	19 (33.9)
2. You use a variety of antibiotics at the same time to increase the effectiveness of Mandarin disease treatment. (negative)	23 (41.8)	3 (5.5)	7 (12.7)	9 (16.4)	13 (23.6)
3. You use only one type of antibiotic in the orchard for a very long period of time. (negative)	5 (9.4)	0 (0.0)	8 (15.1)	9 (17.0)	31 (58.5)
4. You modify or adjust the amount of antibiotics applied to the Mandarin tree by yourself when there is a new pandemic in the orchard. (negative)	17 (31.5)	5 (9.3)	7 (13.0)	9 (16.7)	16 (29.7)
5. You mix or prepare antibiotics in the orchard near water source or on uncovered ground without leakage or leaching prevention. (negative)	20 (36.4)	6 (10.9)	4 (7.3)	9 (16.4)	16 (29.1)
6. You use water from natural sources such as wells, rivers, canals, and ponds for mixing or preparing antibiotics used in the orchard. (negative)	29 (52.7)	4 (7.3)	2 (3.6)	5 (9.1)	15 (27.3)

PPE: Personal protective equipment

Mandarin cultivation (74.2%) and that they are the most effective disease treatment method for Mandarin trees (70.2%). These beliefs about antibiotic use are similar to those expressed by layer and pig farmers in an earlier study on antibiotic use in Thailand (19). The participants in our study also believed that increasing the amount of antibiotics used in Mandarin orchard can increase crop yields (54.7%). This indicates the overuse of antibiotics in Mandarin production. In addition, many of the farmers (47.0%) were uninformed about legal guidelines governing antimicrobials.

In terms of the participants' practices in the Mandarin orchards, they displayed some good habits, for example, wearing appropriate clothes, such as long-sleeved shirts, long pants, and boots to cover their bodies. However, some practices were inappropriate, with 68.9% of the farmers applying antibiotics in the Mandarin orchard, when there were no signs or symptoms of diseases. Almost half of those in the present study (41%) used antibiotics without expert's recommendations or advice. Instead, most farmers (58.9%) used antibiotics in accordance with the orders of the orchard owners. The absence of expert consultation in our study correlates with that of a study on livestock farmers, in which only half of the farmers consulted a veterinarian regarding their decisions to administer antimicrobials to their animals [19] and only half followed the established guidelines [20]. There are no antibiotics currently registered for use in Mandarin production. According to a previous study, without established guidelines on antibiotic concentrations, volumes, frequencies, and withdrawal periods, Mandarin farmers learned antibiotic application techniques based on their own experiences [5].

According to the questionnaire survey, there is evidence of overuse and inappropriate use of

Table-5: The results of antibiotic susceptibility test and SCC*mec* typing of *Staphylococcus aureus* isolates of 282 isolates from 100 Mandarin orchard farmers.

Identification	Antibiotic susceptibility test (Zone, mm)											
	FOX	**P**	**E**	**DA**	**SXT**	**CIP**	**C**	**CN**	**RD**	**TE**	**F**	**LZD**
S. aureus	30	17	30	29	31	30	28	25	32	29	28	27
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	28	16	30	29	31	26	27	28	29	30	23	30
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	27	17	28	28	30	30	26	25	33	31	24	32
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	30	14	27	30	28	29	24	24	33	27	22	28
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	27	14	26	22	18	28	20	22	27	8	23	23
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	28	13	27	28	28	29	23	22	30	9	22	25
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	28	11	25	28	27	28	25	22	33	8	23	25
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	26	15	24	23	17	29	22	22	29	8	22	24
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	27	16	23	23	18	28	24	23	30	18	24	21
	S	R	S	S	S	S	S	S	S	I	S	S
S. aureus	28	15	23	23	18	27	22	22	32	22	23	25
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	28	17	25	26	28	28	26	24	30	27	21	28
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	14	16	29	26	20	29	26	23	29	31	23	30
	R	R	S	S	S	S	S	S	S	S	S	S
S. aureus	22	14	28	24	28	27	24	22	31	28	22	26
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	25	11	24	23	27	21	22	25	27	24	20	25
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	23	14	24	31	30	27	25	25	32	10	22	30
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	26	15	30	26	30	30	26	23	32	10	22	28
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	27	16	30	28	30	30	24	25	32	10	22	31
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	29	15	29	30	30	30	25	28	33	8	23	25
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	28	12	29	24	28	25	28	23	32	10	22	28
	S	R	S	S	S	S	S	S	S	R	S	S
S. aureus	27	14	28	25	26	28	27	24	33	28	21	28
	S	R	S	S	S	S	S	S	S	S	S	S
S. aureus	28	13	29	14	28	28	21	22	28	25	23	25
	S	R	S	S	S	S	S	S	S	S	S	S

S=Susceptible, I=Intermediate, R=Resistant; FOX=Cefoxitin (30 μg), *P*=Penicillin (30 μg), E=Erythromycin (15 μg), DA=Clindamycin (2 μg), SXT=Trimethoprim-sulfamethoxazole (25 μg), CIP=Ciprofloxacin (5 μg), C=Chloramphenicol (30 μg), CN=Gentamicin (10 μg), RD=Rifampin (5 μg), TE=Tetracycline (30 μg), F=Nitrofurantoin (300 μg), LZD=Linezolid (30 μg), CPT=Ceftaroline (30 μg), *S. aureus: Staphylococcus aureus*,

antibiotics in Mandarin orchards. In addition to inadequate knowledge and improper practices, the farmers in the Mandarin orchards tended to be unwarily exposed to antibiotics and unintentionally promoted antibiotic resistance. Out of 100 farmers from whom nasal swabs were obtained, 16 were antibiotic-resistant *S. aureus* carriers. Of the 21 isolates from 16 samples, 21 isolates were resistant to penicillin (21 isolates, 16 samples), tetracycline (eight isolates, six samples), and cefoxitin (one isolates, one sample). All these antibiotics were reported to be used in the orchards by the farmers. Among all isolates, nine isolates from seven samples were multidrug-resistant. Compared to the previous study in the Mandarin orchard of the same location, *Staphylococcus* spp. recovered from nasal samples of Mandarin orchard farmers that were also resistant to ampicillin, which belongs to the penicillin class of drugs and is an indicator of the occurrence of MRSA [8]. The results of this study confirmed the presence of antibiotic-resistant bacteria among Mandarin orchard workers.

This study showed no statistical association between the antibiotic-resistant positive cases and KAP. The high proportion of antibiotic-resistant bacteria in this study may be due to inadequate knowledge and attitudes and improper practices. There is an urgent need to implement the National Strategic Plan on AMR to improve public awareness of antimicrobials and farmers' awareness of the use of antimicrobials in the agricultural sector [4]. In addition, the One

Health approach should be encouraged to identify the major factors responsible for antibiotic-resistant bacteria in Mandarin production facilities in Thailand and the risk of their transmission to humans [21].

This study has several limitations. First, as it included only a small number of orchards (n = 10) and a small number of farmers and farm workers (n = 151), the results may not be generalized to other areas and other Mandarin farmers. However, we had randomly selected the orchards with various sizes to represent every size of orchard. Second, the nasal swab sample size is too small. This might explain why the association between KAP and antibiotic-resistant occurrence was not found. Third, this study was conducted during the COVID-19 pandemic, so nasal swab collection was limited.

Conclusion

This study contributes to the first report on individual KAP regarding antibiotic use among farmers in Mandarin production. The finding in this study implies that educating and encouraging suitable antibiotic use practices in Mandarin production is necessary to minimize the problem of antibiotic resistance. In addition, antimicrobial stewardship and antibiotic use surveillance systems in the plant production sector should be established and implemented through the One Health approach.

Authors' Contributions

WN: Conceptualization, design of the study, and project administration. MH, PO, and AT: Collected data. MH, UW, and WN: Formal analysis, interpreted the data, and visualization. MH and WN: Writing-original draft preparation. PO, AT, and UW: Edited the manuscript. All authors have read, reviewed, and approved the final manuscript.

Acknowledgments

This study was supported by Chiang Mai University Junior Research Fellowship Program, Thailand (Grant number JRCMU2564_063).

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World (Publisher of International Journal of One Health) remains neutral with regard to jurisdictional claims in published map and institutional affiliation.

References

1. Ventola, C.L. (2015) The antibiotic resistance crisis: Part 1: Causes and threats. *P. T.*, 40(4): 277–283.
2. World Health Organization. (2020) Antibiotic Resistance. World Health Organization, Geneva. Available from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance. Retrieved on 01-02-2023.
3. Interagency Coordination Group on Antimicrobial Resistance (2019) No time to Wait: Securing the future from drug-resistant infections: Report to the Secretary-General of the United Nations, World Health Organization.
4. National Health Commission Office. (2023) Thailand National Strategic Plan on Antimicrobial Resistance 2017–2021. National Health Commission Office, Beijing.
5. Chanvatik, S., Donnua, S., Lekagul, A., Kaewkhankhaeng, W., Vongmongkol, V., Athipunyakom, P., Khamlar, S., Prommintara, M. and Tangcharoensathien, V. (2019) Antibiotic use in Mandarin production (*Citrus reticulata* Blanco) in major Mandarin-producing areas in Thailand: A survey assessment. *PLoS One*, 14(11): e0225172.
6. Lekagul, A., Kirivan, S., Tansakul, N., Krisanaphan, C., Srinha, J., Laoprasert, T., Kaewkhankhaeng, W. and Tangcharoensathien, V. (2023) Antimicrobial consumption in food-producing animals in Thailand between 2017 and 2019: The analysis of national importation and production data. *PLoS One*, 18(4): e0283819.
7. Khaneaytip, J., RattanaBuri, S., Promma, C. and Kiatanan, K. (2014) Evaluation of antibiotic use for Greening disease prevention and treatment. Department of Agriculture, Thailand, (in Thai).
8. Rattanapunya, S., Deethae, A., Woskie, S., Kongthip, P. and Matthews, K.R. (2021) Occurrence of antibiotic-resistant *Staphylococcus* spp. in orange orchards in Thailand. *Int. J. Environ. Res. Public Health*, 19(1): 246.
9. Bedekelabou, A.P., Oyetola, D.W., Coulibaly, Z.L., Akinsola, O. and Bada-Alambedji, R. (2022) First assessment of the knowledge, attitudes, and practices of health actors in Togo and Ivory Coast in regard to antibiotic resistance. *Int. J. One Health*, 8(2): 108–123.
10. Sawadogo, A., Kagambèga, A., Moodley, A., Ouedraogo, A.A., Barro, N. and Dione, M. (2023) Knowledge, attitudes, and practices related to antibiotic use and antibiotic resistance among poultry farmers in urban and peri-urban areas of Ouagadougou, Burkina Faso. *Antibiotics* (*Basel*, *Switzerland*), 12(1): 133.
11. Oloso, N.O., Odetokun, I.A., Ghali-Mohammed, I., Fasina, F.O., Olatoye, I.O. and Adetunji, V.O. (2022) Knowledge, attitudes, and risk perception of broiler grow-out farmers on antimicrobial use and resistance in Oyo State, Nigeria. *Antibiotics* (*Basel*, *Switzerland*), 11(5): 567.
12. Dankar, I., Hassan, H. and Serhan, M. (2022) Knowledge, attitudes, and perceptions of dairy farmers regarding antibiotic use: Lessons from a developing country. *J. Dairy Sci.*, 105(2): 1519–1532.
13. Dohoo, I. and Emanuelson, U. (2021) The use of item response theory models to evaluate scales designed to measure knowledge of, and attitudes toward, antibiotic use and resistance in Swedish dairy producers. *Prev. Vet. Med.*, 195: 105465.
14. Sayasonthi, B. and Paiboonkunko, K. (2021) Knowledge attitudes and practices regarding antibiotic use among dairy farmers in Kamphaeng Saen district, Nakhon Pathom province. *KKU Vet. J.*, 31(1): 13–28.
15. Dyar, O.J., Zhang, T., Peng, Y., Sun, M., Sun, C., Yin, J., Ding, L., Sun, C., Wang, Y., Sun, Q., Greko, C. and Lundborg, C.S. (2020) Knowledge, attitudes and practices relating to antibiotic use and antibiotic resistance among backyard pig farmers in rural Shandong province, China. *Prev. Vet. Med.*, 175: 104858.
16. Caudell, M.A., Dorado-Garcia, A., Eckford, S., Creese, C., Byarugaba, D.K., Afakye, K., Chansa-Kabali, T., Fasina, F.O., Kabali, E., Kiambi, S., Kimani, T., Mainda, G., Mangesho, P.E., Chimpangu, F., Dube, K., Kikimoto, B.B., Koka, E., Mugara, T., Rubegwa, B. and Swiswa, S. (2020) Towards a bottom-up understanding of antimicrobial use and resistance on the farm: A knowledge, attitudes, and practices survey across livestock systems in five African countries. *PLoS One*, 15(1): e0220274.

17. Department of Medical Sciences. (2017), Chapter 4: Managing the Specimens Processing. In Good Clinical Laboratory Practices Guidelines, 2021. Ministry of Public Health, Department of Medical Sciences, India.
18. Rongsanam, P., Yano, T., Yokart, W., Yamsakul, P., Sutammeng, S., Udpaun, R., Pichpol, D., Tamdee, D. and Anukool, U. (2020) Acquisition Risk Factors of the SCC*mec* IX-Methicillin-Resistant *Staphylococcus aureus* in Swine production personnel in Chiang Mai and Lamphun provinces, Thailand. *Antibiotics* (*Basel, Switzerland*), 9(10): 651.
19. Nuangmek, A., Rojanasthien, S., Patchanee, P., Yano, T., Yamsakul, P., Chotinun, S. and Tadee, P. (2018) Knowledge, attitudes and practices toward antimicrobial usage: A cross-sectional study of layer and pig farm owners/ managers in Chiang Mai, Lamphun, and Chonburi provinces, Thailand, May 2014 to February 2016. *Korean J. Vet. Res.*, 58(1): 17–25.
20. Gebeyehu, D.T., Bekele, D., Mulate, B., Gugsa, G. and Tintagu, T. (2021) Knowledge, attitude and practice of animal producers towards antimicrobial use and antimicrobial resistance in Oromia zone, north eastern Ethiopia. *PLoS One*, 16(5): e0251596.
21. Sudatip, D., Chasiri, K., Kritiyakan, A., Phanprasit, W., Thinphovong, C., Tiengrim, S., Thamlikitkul, V., Abdallah, R., Baron, S.A., Rolain, J.M., Morand, S., Hilty, M. and Oppliger, A. (2021) A One Health approach to assessing occupational exposure to antimicrobial resistance in Thailand: The FarmResist project. *PLoS One*, 16(1): e0245250.t

****