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Prevalence and antibiogram study of *Salmonella* and *Staphylococcus aureus* in poultry meat

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PEER REVIEW

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Comments
The study is up-to-dated and shows the importance of monitoring the common foodborne disease vehicles. It shows the presence of *Salmonella* and *S. aureus* in poultry meat with drug resistance isolates. The use of antibiotic in animal farming is one of the causes of drug resistance development in bacterial isolates.
(Details on Page 167)

ABSTRACT

Objective: To evaluate the presence and antibiogram pattern of *Salmonella* and *Staphylococcus aureus* (*S. aureus*) in retail poultry meat products. **Methods:** Foodborne pathogens (*Salmonella* and *S. aureus*) were isolated from poultry meat and confirmed with the help of biochemical and immunological test. Antibiogram of the isolates were examined by following CLSI methods. **Results:** A total number of 209 poultry meat samples were collected and studied in this study. Out of which, 5.26% were found contaminated with *Salmonella* while 18.18% were found contaminated with *S. aureus*. All the *Salmonella* and *S. aureus* isolates were found resistant to at least one antibiotic. About 72.72% of the *Salmonella* isolates showed resistance to tetracycline, while *S. aureus* isolates were also found highly resistant to tetracycline equal to 44.73%. One of the *Salmonella* isolates showed multi-drug resistance to almost six antibiotics out of nine antibiotics used in the study. Multidrug resistant *S. aureus* isolates were also found in the study. **Conclusions:** The study confirmed the presence of *Salmonella* and *S. aureus* in retail poultry meat. It is a potential threat to consumer health. To reduce the risk of contamination, good hygiene practices are necessary from processing to storage.

KEYWORDS
Antibiogram, *Salmonella*, *Staphylococcus aureus*, Food safety, Poultry meat

1. Introduction

Foodborne diseases and poisoning are the widespread and great public health concerns of the modern world. Both developed and developing countries are largely affected by foodborne infections. Foodborne diseases not only affect people's health and well-being, but also have economic impacts on individuals and the countries[1]. It has been estimated that, each year, approximately 22.8 million cases of Salmonellosis occurred with death toll of 37 600 in South East Asia[2]. The foodborne pathogens are responsible to impose a substantial burden of infection in the developed countries, while the impact in case of developing countries is higher. It reduces markedly social and economic productivity of the countries[3]. Amongst the foodborne pathogens, *Salmonella* and *Staphylococcus aureus* (*S. aureus*) are the most common and frequent pathogens responsible for food poisoning and food related infections[4,5]. *Salmonella enterica* is a leading cause of enteric diseases in human and animal with millions of illness worldwide, whereas the non-typhoidal *Salmonella* species as a zoonotic agent are also predominantly associated with foodborne infections[2,6,7]. *Salmonella* species in foods of animal origin are most frequently considered to be associated with the foodborne pathogen outbreaks. Improper cooking, inadequate

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storage, cross-contamination and use of raw ingredients in the preparation of food are the most common factors contributing to outbreaks[2,8-10].

In the majority of foodborne infections, it is not possible to identify the food vehicle. Poultry meat is considered as the most commonly reported foodborne pathogens vehicle followed by the red meat[11]. *S. aureus* is responsible for causing a variety of animal diseases such as mastitis, arthritis and urinary tract infections and a prominent cause of food poisoning due to poor hygienic practices. *S. aureus* related food poisoning is the third largest cause of food related illness worldwide[5,12,13]. Enterotoxin production is the lethal weapon of this pathogen. Its food poisoning is commonly associated with fresh and ready-to-eat foods particularly meat products[5]. It is more difficult to treat multidrug resistant *S. aureus* as compare to drug susceptible strains. Methicillin resistant *S. aureus* (MRSA) is responsible for a huge number of hospital acquired infections[14,15]. MRSA is equally associated with human and veterinary infections[16].

The prevalence of antimicrobial drug resistance among foodborne pathogens is increased due to its use in human therapy and animal farming for therapeutic and prophylactic purposes. Multidrug resistant *Salmonella* are frequently isolated from food sources in South East Asia. It's hard to treat the infections caused by multidrug resistant bacteria as compare to normal one. Such strains are more dangerous and of great food safety concern[2,10,17]. The increase in the prevalence of multi-drug resistant *Salmonella*, particularly resistance to fluoroquinolones and third-generation cephalosporins are an emerging problem worldwide[9]. Monitoring the presence of foodborne pathogens in foods is the primary tool for the implementation of food safety systems. It is necessary to monitor the prevalence and antimicrobial resistance of foodborne pathogens for effective food safety planning and targeted interventions[7]. Food plays an important role in the transfer of antibiotic resistance in term of antibiotic residues or transfer of resistant genes from food microflora to pathogenic bacteria[18]. The objective of this study is to reveal the presence of potential foodborne pathogens *Salmonella* and *S. aureus* in the retail poultry meat in Thailand and furthermore to evaluate the antimicrobial resistance and sensitivity pattern of these pathogens.

2. Materials and methods

A detail study was carried out to reveal the prevalence and antibiogram of *Salmonella* and *S. aureus*, in raw poultry meat in Bangkok area of Thailand.

2.1. Sampling

Fresh poultry meat (n=209) was collected randomly from different markets in greater region of Bangkok Thailand. Sampling box containing ice pads was used for carrying the samples from market to laboratory maintaining low temperature. All the samples were processed in Food Engineering and Bioprocess Technology (FEBT), laboratory at the Asian Institute of Technology (AIT) Thailand. The entire collected samples were processed within 4 h after collection from the vendors. The samples were collected randomly, and each market was chosen according to the convenience and distance to the laboratory. Samples were marked with identification code, like (FTT1) with respect to area, date and time. Sampling criteria was limited to 250 g of one sample per vender in the open market and one packet of packed poultry meat in supermarket. Samples were not limited to any specific part.

2.2. Control strain

S. aureus (TISTR 029) and *Salmonella* Typhimurium (TISTR 292) were used as control strains for media and kits validation.

2.3. Bacterial culturing and isolation

Meat with hard pieces or bony samples was first trimmed with sterile knife aseptically. Samples (25 g) were transferred to 225 mL of buffered peptone water (BPW) and mixed (IKA Labortechnik Germany) for 10 min at 120 r/min, the mixture sample were incubated at 37 °C for 18-24 h. Part of inoculated BPW was transferred to two separate enrichment media for isolation of *Salmonella* and *S. aureus*.

2.4. Isolation of *S. aureus*

Pre-incubated samples (0.1 mL) in BPW were spread on the surface of Baird-Parker agar medium (Himedia, India) supplemented with Egg Yolk Tellurite Emulsion (Himedia) and Mannitol salt agar (MSA) (Himedia) a selective media for *S. aureus* and incubated further at 37 °C for 24-48 h. Black colonies surrounded by opaque halo on Baird-Parker agar and yellow colonies on MSA were considered presumptive *S. aureus*, confirmed with the help of Gram's staining and other biochemical tests. Gram's positive, catalase and coagulase positive *S. aureus* isolates were confirmed finally with the help of Pastorex Staph Plus kit (Bio-Rad, USA). The isolates were stored on nutrient broth containing 20% (v/v) glycerol at -20 °C for further study. The methicillin susceptible and resistant (MSSA and MRSA) isolates were identified with the help of oxacillin disk diffusion test[19].

2.5. Isolation of *Salmonella*

One milliliter of pre-inoculated BPW were transferred to 10 mL of selective enrichment media rappaport vassiliadis soyabean meal broth (RVSM) (BioMark, India) and tetrathionate broth (TTB) (BioMark) and incubated further at 37 °C for 24 h. On the following day, two loopful of RVSM and TTB were transferred to bismuth sulfite agar

(BSA) (Himedia) and xylose lysine deoxycholate agar (XLD) (Difco, USA) and further incubated at 37 °C for 24-48 h to isolate visible colonies of *Salmonella* species. Pink colonies with black centers on XLD agar and brown, gray or black colonies with metallic sheen on BSA were supposed to be *Salmonella*. Positive colonies were then transferred to triple sugar iron agar (Himedia) and lysine iron agar (Himedia) and further confirmed by submitting to API 20E kit (Biomerieux, France) along with api web and with the help of polyvalent antisera "O" and "H" (Serosystem, Clinag, Thailand).

2.6. Antibiogram of Salmonella and S. aureus

Antimicrobial susceptibility pattern of *Salmonella* and *S. aureus* isolates were determined by using disk diffusion assay following the guidelines of Clinical and Laboratory Standard Institute[20]. The pre-incubated 24 h cultures of *Salmonella* and *S. aureus* were diluted in sterile buffer peptone water and matched with the 0.5 MacFarlane turbidity standards to get 1×10^8 CFU/mL as total count. Bacterial suspensions were spread on mueller-hinton agar (Merck, Germany). The antibiotic discs were placed over the lawn and incubated at 37 °C for 18-24 h. The clear zone around each antibiotic disc was measured in millimeter. The following selected commonly in use antibiotics were used: Oxacillin (1 μg), Ciprofloxacin (5 μg), Ampicillin (10 μg), Tetracycline (30 μg), Sulfamethoxazole/Trimethoprim (25 μg), Gentamicin (10 μg), Chloramphenicol (30 μg), Nalidixic acid (30 μg), Streptomycin (10 μg) and Kanamycin (30 μg) (Oxoid, UK).

3. Results

In the prevalence study, out of total two hundred and nine poultry meat samples, eleven (5.26%) were found contaminated with *Salmonella* species as conformed by biochemical and immunological testing kits. The presence of *S. aureus* in poultry meat was found in 38 out of total 209 samples (18.18%). All the samples were conformed primarily with the help of its growth characteristics on selective media and then with the help of biochemical and immunological testing kits. All the conformed isolates were then submitted to antibiogram study to evaluate its antibiotic sensitivity and resistance patterns.

All the *Salmonella* and *S. aureus* isolates were exposed to different antibiotics and its antimicrobial drug response were studied as shown in (Table 1) for *S. aureus* and in (Table 2) for *Salmonella*. Out of all eleven, 45.45% of the *Salmonella* isolates showed resistance to streptomycin, whereas 27.27% of the isolates were found resistance to ciprofloxacin, ampicillin and sulfamethoxazole/trimethoprim. Most of the *Salmonella* isolates (about 73%) were resistant to tetracycline. While the lowest resistance of the isolates 18.18% were against chloramphenicol. Resistance to gentamicin and kanamycin were about 36.36%. The resistance against nalidixic acid was found 54.54%. It has been observed that all isolate showed resistance to at least one antibiotic, while the majority of the isolates showed resistance to three different antibiotics. Some of the isolates show multidrug resistance to five and six antibiotics used in the study.

Table 1

Antibiogram profile of *S. aureus* isolates of poultry meat.

Isolates ID	Resistance	Sensitive
FBC1	Oxa Cip Amp Chl	Str Tet Sxt Gen
FBC2	Oxa Amp Sxt	Str Cip Tet Gen Chl
FBK2	Oxa Str Amp	Cip Tet Sxt Gen Chl
FBK3	Tet Gen	Oxa Str Cip Amp Sxt Chl
FTT6	Tet Chl	Oxa Str Cip Amp Sxt Gen
FTT7	Sxt	Oxa Str Cip Amp Tet Gen Chl
FLN3	Amp Tet	Oxa Str Cip Sxt Gen Chl
FLN4	Chl	Oxa Str Cip Amp Tet Sxt Gen
FTT8	Amp Sxt	Oxa Str Cip Tet Gen Chl
FBC7	Amp Tet	Oxa Str Cip Sxt Gen Chl
FTT11	Amp	Oxa Str Cip Tet Sxt Gen Chl
FBC10	Str Sxt Gen	Oxa Cip Amp Tet Chl
FBC11	Amp Tet Chl	Oxa Str Cip Sxt Gen
FTT15	Amp	Oxa Str Cip Tet Sxt Gen Chl
FBC14	Tet Sxt	Oxa Str Cip Amp Gen Chl
FLN7	Amp	Oxa Str Cip Tet Sxt Gen Chl
FTT17	Amp Tet	Oxa Str Cip Sxt Gen Chl
FBC15	Sxt	Oxa Str Cip Amp Tet Gen Chl
FTT24	Cip Chl	Oxa Str Amp Tet Sxt Gen
FLN13	Amp Tet	Oxa Str Cip Sxt Gen Chl
FTT29	Str Tet Gen	Oxa Cip Amp Sxt Chl
FLN18	Amp Sxt	Oxa Str Cip Tet Gen Chl
FLN19	Amp Tet	Oxa Str Cip Sxt Gen Chl
FLN20	Amp	Oxa Str Cip Tet Sxt Gen Chl
FLN24	Tet	Oxa Str Cip Amp Sxt Gen Chl
FTT37	Amp Tet	Oxa Str Cip Sxt Gen Chl
FTT37	Str Sxt	Oxa Cip Amp Tet Gen Chl
FTT38	Tet	Oxa Str Cip Amp Sxt Gen Chl
FTT41	Amp	Oxa Str Cip Tet Sxt Gen Chl
FLN28	Amp Chl	Oxa Str Cip Tet Sxt Gen
FTT44	Str Gen	Oxa Cip Amp Tet Sxt Chl
FBC26	Sxt	Oxa Str Cip Amp Tet Gen Chl
FLN31	Cip Amp Tet	Oxa Str Sxt Gen Chl
FTT45	Str Sxt Gen	Oxa Str Cip Sxt Chl
FBC30	Amp Tet	Oxa Str Cip Sxt Gen Chl
FTT50	Amp Tet Chl	Oxa Str Cip Sxt Gen
FLN35	Str Sxt	Oxa Cip Amp Tet Gen Chl
FLN36	Tet Chl	Oxa Str Cip Amp Sxt Gen

Oxa (Oxacillin 1 μg) Str (streptomycin 10 μg), Cip (ciprofloxacin 5 μg), Amp (ampicillin 10 μg), Tet (tetracycline 30 μg), Sxt (sulfamethoxazole/trimethoprim 25 μg), Gen (gentamicin 10 μg), Chl (chloramphenicol 30 μg)

The antimicrobial sensitivity and resistance pattern of all *S. aureus* isolates were studied and showed in (Table 1). Out of the total thirty eight isolates, three showed resistance to oxacillin and was termed as methicillin resistance *S. aureus*, makes 7.89% part of the total isolates. The same percentage of *S. aureus* was found resistant to ciprofloxacin, whereas the resistance against gentamicin, streptomycin and chloramphenicol were recorded 13.15%, 18.42% and 21.05% respectively. The isolates, about 28.94% showed resistance to sulfamethoxazole/trimethoprim. The highest 44.73% and 55.26% of the isolates were resistant to tetracycline and ampicillin respectively.

Table 2
Antibiogram profile of *Salmonella* isolates of poultry meat.

Isolates ID	Resistance	Sensitive
FTT2	Tet	Str Cip Amp Sxt Gen Chl Nal Kan
FBC5	Str Cip Amp Tet Chl	Sxt Gen Nal Kan
FTT16	Tet Gen Nal	Str Cip Amp Sxt Chl Kan
FBC12	Str Nal Gen	Cip Amp Tet Sxt Chl Kan
FLN11	Str Tet Sxt kan	Cip Amp Gen Chl Nal
FTT31	Str Cip Amp	Tet Sxt Gen Chl Nal Kan
FLN22	Tet Sxt Nal	Str Cip Amp Gen Chl Kan
FTT38	Str Tet Gen Chl Nal Kan	Cip Amp Sxt
FLN31	Tet Sxt Nal	Str Cip Amp Gen Chl Kan
FLN32	Cip Gen Kan	Str Amp Tet Sxt Chl Nal
FTT51	Amp Tet Nal Kan	Str Cip Sxt Gen Chl

Str (streptomycin 10 μg), Cip (ciprofloxacin 5 μg), Amp (ampicillin 10 μg), Tet (tetracycline 30 μg), Sxt (sulfamethoxazole/trimethoprim 25 μg), Gen (gentamicin 10 μg), Chl (chloramphenicol 30 μg), Nal (nalidixic Acid 30 μg), Kan (kanamycin 30 μg)

4. Discussion

This is well established facts that contaminated food is the main source of transmission for pathogenic bacteria. It is the major cause of enteric diseases in developing countries and is a major cause of mortality and morbidity[10]. Poultry meats as a main source of foodborne infections have great impact in food safety.

In this study, the presence of *Salmonella* and *S. aureus* was found about 5.25% for *Salmonella* and 18.18% for *S. aureus* in retail poultry meat with some multi–drug resistance isolates. Pathogenic bacteria like *Salmonella* and *S. aureus* from food sources have been confirmed by different authors all over the world. Ellerbroek *et al.* reported 13% prevalence of *Salmonella* isolates from imported chicken carcass in Bhutan[21]. While Minami *et al.* reported 25% prevalence in different types of meat including chicken in Thailand[22]. Their study shows that *Salmonella* is more prevalent in the case of chicken or poultry meat. Fernández *et al.* in their study in 1993 and 2006 recorded 22.7% prevalence of *Salmonella* in poultry meat samples in Spain[7]. Zhao *et al.* reported 4.2% prevalence of *Salmonella* contamination in chicken meat in a similar study in USA[23]. That is in agreement with our findings. Seza and Ayla reported 29.3% prevalence of *Salmonella* in poultry meat[24] .

S. aureus has a wide range of habitats including human body parts, which may contaminate the food. It is considered being one of the most important foodborne illnesses causing pathogenic species. It's present in food indicates poor hygiene and improper storage conditions[25]. De Boer *et al.* reported 11.9% MRSA prevalence in meat whereas 16% in chicken meat alone[26]. Gundogan *et al.* reported 53% of *S. aureus* contamination of meat and chicken samples[25]. Atanassova *et al.* found 51.1% *S. aureus* contamination in raw pork meat by PCR detection while he claimed 57.7% *S. aureus* contamination by using classical microbiological procedures[27]. Heo *et al.* reported 11% *S. aureus* prevalence in meat, while Lee reported 13% *S. aureus* presence in poultry meat of Korea[15,28].

Emerging drug resistance in the foodborne bacterial isolates is a great public health concern. Ellerbroek *et al.* reported 27% and 34.8% resistance against kanamycin and gentamicin respectively to *Salmonella* isolates in a similar study which is in compliance of our study[21]. In a similar study in Thailand by Minami *et al.*, majority of *Salmonella* isolates exhibit resistance to tetracycline and streptomycin, and a number of multi–drug resistant *Salmonella* were reported, showed resistance to ampicillin, tetracycline, streptomycin, gentamicin, chloramphenicol and kanamycin[22]. Dione *et al.* reported that high rate of *Salmonella* resistance was counted against sulfamethoxazole/trimethoprim (75.9%), tetracycline (74.7%) and streptomycin (73.9%)[29]. Extensive use of antibiotic in animals contributed to increase the resistance against antibiotics in different bacterial strains over the last 30 years. *Salmonella* enterica strains DT104 is one of the multi–drug resistance group, show resistance to ampicillin, streptomycin, chloramphenicol, sulfonamides and tetracycline[30]. Chuanchuen and Padungtod found that resistance genes were responsible for almost 78% of the *Salmonella* enterica isolates from poultry and pig meat[31]. All the strains find resistant to three or more than three antibiotics, blaTEM, cmlA, tetA, dfrA12, sul3 aadA1 genes were found in majority of the strains, showed resistance to ampicillin (87%), chloramphenicol (63%), tetracycline (60%), trimethoprim (42%), sulphonamides (42%) and streptomycin/spectinomycin (61%)[31]. A serovar Heidelberg and a serovar Newport of *Salmonella* from organic chicken were found resistant to nine antibiotics, including cefoxitin, ampicillin, amoxicillin–clavulanic acid, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, ceftiofur and cephalothin[32]. In a study, Aslama *et al.* found that 29% *Salmonella* isolates from retail meats in Canada are susceptible to all antimicrobial used (amoxicillin–clavulanic acid, ceftiofur, ceftriaxone, ciprofloxacin, amikacin, ampicillin, cefoxitin, gentamicin, kanamycin, nalidixic acid, streptomycin, trimethoprim/sulfamethoxazole, chloramphenicol, sulfisoxazole, tetracycline) and no resistance were found to amikacin, ciprofloxacin and nalidixic acid in any *Salmonella* isolate[33]. They confirmed that 29% of the *Salmonella* isolates from chicken have no resistance genes.

Ellerbroek *et al.* reported 92.2% resistance to tetracycline, while 41.5% and 49.4% resistance to chloramphenicol and ciprofloxacin of *Salmonella* isolates from chicken meat[21]. They also reported 40.7%, 36.0% and 28% resistance to ampicillin, streptomycin, and nalidixic acid respectively. *Salmonella* serovar Heidelberg are resistant to ceftiofur, streptomycin, tetracycline, sulfamethoxazole, chloramphenicol and trimethoprim–sulfamethoxazole. This strain of *Salmonella* derived from poultry products is specifically important due to its ability to cause extra intestinal infection like septicemia[34].

Resistant *S. aureus* is more frequently associated with nosocomial infections in hospitals, and even in intensive care units (ICU) in many of the developing countries[35]. In antibiogram study for *S. aureus* isolates from meat origin, Heo *et al*. reported 92.9% and 50% resistance of tetracycline and ampicillin respectively[15]. Otalu *et al*. reported 100% resistance in *S. aureus* isolates from poultry meat against tetracycline and 61.5% against methicillin in Nigeria[36]. They reported 46.2% and 15.4% resistance against chloramphenicol and ciprofloxacin whereas, 38.5% against gentamicin and sulfamethoxazole/trimethoprim. Multidrug resistant *S. aureus* have been reported several times[37]. Achi and Madubuikereported 59.2% tetracycline to *S. aureus* isolates from ready-to-eat food[13]. Extensive uses of these antibiotics are thought to be the major cause of drug resistance in foodborne pathogens[36].

In this study, it has been found that foodborne pathogens *Salmonella* and *S. aureus* are largely associated with the poultry meat. Presence of drug resistant foodborne pathogen particularly *Salmonella* is a great public health concern. It is revealed in the study that the presence of *Salmonella* and *S. aureus* is a potential threat to consumer health. The development of drug resistance by these common pathogens is a matter of concern in food safety. It seems that the majority of commonly used antibiotics are not effective against *Salmonella* and *S. aureus*. Biopreservation and bio-control strategies should be adopted to cope with the problems related to the chemical preservatives and antibiotics used in animal farming and food processing. Maintenance of good hygiene practices in meat processing industries can reduce the chances of contamination. Searching and developing novel antibiotic is necessary for the treatment of multi-drug resistant bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Foodborne disease is a major public health problem, each year hug number of hospitalization and deaths occur due to the use of contaminated food. Each year, approximately 22.8 million cases of Salmonellosis with a death toll of 37 600 occurred in South East Asia. *Salmonella* is the leading cause of foodborne diseases in human and animals. Whereas food positioning related to *S. aureus* is counting to be the third largest cause of food related illness worldwide.

Research frontiers

Detailed study has been performed to know the prevalence of two important foodborne pathogens in the the poultry meat. *Salmonella* and *S. aureus* have been isolated from meat samples. The drug resistance pattern of the isolates is listed in the article.

Related reports

Number of researcher studied the prevalence of foodborne pathogens. *Salmonella* and *S. aureus* is of great concern. Fernández *et al*. reported 22.7% *Salmonella's* presence in poultry meat in Spain. Lee reported 13% *S. aureus's* presence in poultry meat of Korea.

Innovations and breakthroughs

The data in this article show the details of the pathogenic bacteria's presence in the poultry meat. It is also revealed here that multi-drug resistance (MDR) bacteria are present in poultry meat. Cross contamination and infection from foodborne MDR *Salmonella* and *S. aureus* can lead to prolonging illness and death in some cases.

Applications

The date in the article is helpful for the effective planning and implementation of food safety systems for the preservation and control of food related pathogenic bacteria. The article shows that poultry meat is a vehicle of bacterial transfer and plays an important role in diseases prevalence.

Peer review

The study is up-to-dated and shows the importance of monitoring the common foodborne disease vehicles. It show the presence of *Salmonella* and *S. aureus* in poultry meat with drug resistance isolates. The use of antibiotic in animal farming is one of the causes of drug resistance development in bacterial isolates.

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