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Analysis of Pathogenic Bacteria in Ready-to-eat Fried Chicken in the Jember University Campus Area

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ABSTRACT

Poultry meat is a food that is widely consumed in various forms, but it is also a reservoir of bacteria. This study aims to isolate and identify the type of contaminant bacteria in fried chicken sold in the Jember University campus area. This research is a laboratory epidemiological survey research, in January - March 2021. 79 fried chicken samples came from 27 stalls along Jalan Kalimantan, Jalan Jawa, Jalan Riau and Jalan Mastrip Jember. Isolation and identification of bacteria using chromogenic media, namely Mannitol Salt Agar (MSA) and Salmonella Chromogenic Agar (SCA). The identification of bacteria based on the color of the growing bacterial colonies was then confirmed with API E 20 media. The results showed that 98.7 % of fried chicken samples contained at least 1 type of bacteria. The types of bacteria that contaminate fried chicken are *Escherichia coli* (98.8 %), *Staphylococcus aureus* (94.9 %), *Salmonella typhimurium* (26.5 %), *Salmonella typhi* (8.8 %), and *Proteus spp*. (2.5 %). Thus, consumers' proper hygiene and safety practices have been suggested as the main intervention and need to be followed up with regular surveys to assess behavioral changes and reduce knowledge gaps.

Keywords: ready to eat, fried chicken, foodborne diseases

INTRODUCTION

Foodborne diseases (FBD) have become a significant public health problem worldwide due to the increasing incidence of foodborne diseases over the past 20 years (Lee et al., 2017; Ma et al., 2020). However, it is difficult to estimate the global incidence of FBD disease because some cases are not reported, especially in developing countries, including Indonesia. It is estimated that 600 million approximately 1-10 people fall ill after eating contaminated food. As many as 420 people die each year, resulting in the loss of 33 million years of healthy life (World Health Organization (WHO)., 2020). Pathogens that often cause FBD are microorganisms (*Escherichia coli* O157:H7 bacteria, *Salmonella enterica*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Bacillus cereus*, and *Shiga-toxin producing E. coli strains* (non-O157 STEC), and *Vibrio spp*. (Zhao et al., 2014).

The eating habits of the community, especially students, have significantly changed in recent years. Today, eating out is on the rise, even though food is usually handled, arranged, and sold at roadside eateries and other open spaces that are generally unhygienic. The factors driving people to eat out of the home may be women's participation in the work sector, the tendency to change lifestyles, working overtime, being away from home while working, rapid urbanization, and longing for further education and research (Zaghloul, 2014). This condition increases the risk of consuming food contaminated by pathogenic microbes and can cause gastrointestinal infections (Susanna et al., 2012; Van Kampen et al., 1998).

Some of the pathogens that can be found in crispy chickens include *S. typhimurium* DT104, E. coli O157:H7, *L. monocytogenes*, *S. aureus*, and *B. cereus*, *Klebsiella spp*., *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Campylobacter* (Akter et al., 2019; Aung et al., 2018; Haftay et al., 2018; Kayalvizhi & Antony, 2011). Previous research in Singapore found contamination with *Campylobacter* and *E Coli* in boiled chicken (Aung et al., 2018), in Benin City, China, found E coli bacterial contamination in crispy chicken (OP et al., 2016), as well as in Bangladesh (Hessel et al., 2019). Most of these pathogens cause clinical manifestations in diarrhea and fever, which under certain conditions can cause severe dehydration or systemic infectious diseases such as sepsis which has a high mortality rate, especially in vulnerable populations such as children aged < 5 years and the elderly (WHO, 2015).

Research on microbial contamination in ready-to-eat food, especially fried chicken, is still few in Indonesia. Research conducted in Jember on broiler chicken showed contamination with E Coli bacteria, and some isolates were resistant to tetracycline antibiotics (Putri et al., 2018). However, research on Balinese food sequences made from the raw chicken after the cooking process did not find any bacterial contamination and was safe for consumption (Sudiarta et al., 2019).

Enactment of Law no. 7 concerning food in 1996 is a step forward that the government has achieved to protect consumers and producers of healthy, safe, and halal food. The general description of the state of food safety for the last three years is: (1) There are still distributions of food products that do not meet the requirements; (2) There are still many cases of food poisoning; (3) The responsibility and awareness of producers and distributors regarding food safety are still low.

Jember Regency is one of the regencies in East Java Province, which has a Jember University campus with around 20,000 students from various regions in Indonesia. The existence of these students is an opportunity for the local community to open food stalls around the campus area. Fried chicken is one of the favorite foods of today's youth. As the younger generation, students must get safe food for consumption to support health and success in studying in college. UNEJ's research strategic plan related to agricultural biotechnology and health, especially roadmaps and research outputs related to research with Food Safety that supports public health.

Recently, fast food consumption has increased widely, and the fast-food sector is becoming an important industry. Due to improper handling and management, such foods can quickly become contaminated with pathogenic microorganisms, which often cause foodborne illness and even death. Therefore, this study was conducted to evaluate the microbiological quality of ready-to-eat fried chicken samples. Based on this background, it is necessary to detect, identify and test the sensitivity of bacteria isolated from crispy fried chicken sold in the Jember University campus area.

MATERIALS AND METHOD

The Ethics Commission has approved this research of the Faculty of Medicine, the University of Jember, with letter no: 1447/H25.1.11/KE/2020, carried out at the Microbiology Laboratory, Faculty of Medicine, the University of Jember in January-March 2021.

Samples were collected randomly from 25 warungs or merchants of five times and five modern shops in the Jember University campus area. Each sample was stored in a sterile polythene bag and brought to the Microbiology laboratory of the Faculty of Medicine, University of Jember. 25-gram fried chicken soaked in 225 mL sterile distilled water for 15 minutes, stirred vigorously, then blended and analyzed further. 1 ose suspension of fried chicken was taken to be planted on Mannitol Salt Agar (MSA) and Salmonella chromogenic Agar (SCA) media by scratching and incubated for 24 hours at 37 0 C. On day 2, the growth of bacterial colonies was observed. There are three colors of colonies that may grow, namely blue (E. coli), magenta red (Salmonella), and clear yellow (Proteus). The culture results were then characterized by planting on API E 20 media and then confirmed using Gram staining.

RESULT AND DISCUSSION

Foodborne disease is caused by pathogenic bacteria such as *E. coli*, *Salmonella*, *Saurus*, *Proteus spp*. It has become a severe public health problem worldwide. This pathogen is transmitted mainly through the consumption of contaminated food, and therefore the presence of such microorganisms in meat has relevant public health implications (Haasan et al., 2020). From 27 stalls, there were 79 samples of fried chicken consisting of 26 thighs, 26 wings, and 27 fried chicken breasts. Almost all samples (98.7%) detected at least 1 type of pathogenic bacteria (Table 1). The results of this study are almost the same as previous studies in Tennessee, which found that 95.2% contained enterobacteria in chicken, beef, and turkey

(Kilonzo-Nthenge et al., 2013). This can happen because harmful microorganisms are widespread in soil, water, animals, and food handlers. These microorganisms spread on hands, clothing, utensils, and cutting boards; slight contact will transfer to meat and chicken diets and cause foodborne illness. Raw foods, especially meat, poultry, and juices, contain harmful microorganisms transferred to other foods during preparation and storage (WHO, 2015). Based on the bacterial identification test results using MSA and SCA media, five bacteria were found in fried chicken, namely *S. aureus*, *E. coli*, *S. typhi*, *S. Typhimurium*, and *Proteus spp.* (Table 2 and Figures 1 and 2).

Table 1. Prevalence of bacterial contaminants in fried chicken sold in the Jember University campus area

Fried chicken	n	Positive	Percentage
Thigh	26	26	100%
Wing	26	25	96.2%
Chest	27	27	100%
Total	79	78	98.7%

Table 2. Results of Isolation and Identification of bacterial contaminants in fried chicken sold in the Jember University campus area

Stall	Types of bacteria		
	Thigh	Wing	Chest
1	*E. coli, S. typhimirium, Proteus sp*	*Negatif*	*Proteus sp*
2	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S.aureus*	*E. coli, S. typhimirium, S. aureus*
3	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
4	*E. coli, S aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
5	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
6	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
7	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
8	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
9	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*
10	*E. coli, S typhi, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*
11	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*
12	*E. coli, S. aureus*	*E. coli, S. typhi, S. aureus*	*E. coli, S. aureus*
13	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
14	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*
15	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhi*
16	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*
17	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*
18	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*
19	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. typhimirium, S. aureus*
20	-	*E. coli, S. aureus*	*E. coli, S. aureus*
21	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
22	*E. coli, S. aureus*	-	*E. coli, S. aureus*
23	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. aureus*
24	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*	*E. coli, S. aureus*
25	*E. coli, S. aureus*	*E. coli, S. aureus*	*E. coli, S. typhimirium, S. aureus*
26	*E. coli, S. aureus*	*E. coli, S. typhi, S. aureus*	*E. coli, S. aureus*
27	*E. coli*	*E. coli, S. aureus*	*E. coli, S. typhi, S. aureus*

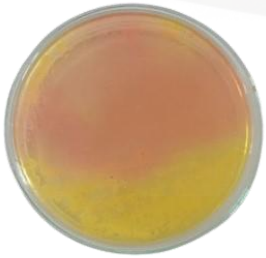

Figure 1. Culture results on MSA

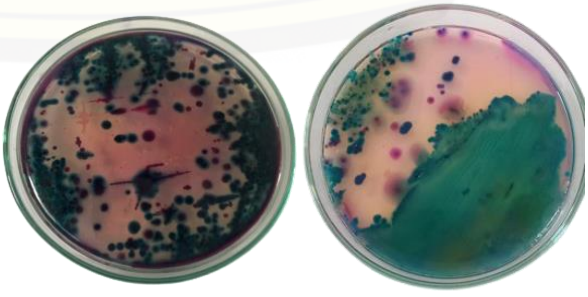

Figure. 2 Culture results on SCA

Table 3. Prevalence of contaminant bacteria based on fried chicken organ

Fried chicken	Types of contaminants bacteriae														
	S. aureus			*E. coli*			*S. typhimirium*			*S. typhi*			*Proteus spp.*		
	Neg	Pos	%	Neg	Pos	%	Neg	Pos	%	Neg	Pos	%	Neg	Pos	%
Thigh	2	24	92.3	0	26	100	19	7	30.4	25	1	4.3	25	1	4.3
Wing	1	25	96.3	0	26	100	21	5	21.7	23	3	11.5	26	0	0.0
Chest	1	26	96.2	1	26	96.2	18	9	33.3	24	3	11.1	26	1	4.2
Total	4	75	94.9	1	78	98.8	58	21	26.5	72	7	8.8	77	2	2.5

From 79 samples of fried chicken found *Escherichia coli* (98.8%), *Staphylococcus aureus* (94.9%), *Salmonella Typhimurium* (26.5%), *Salmonella typhi* (8.8%), and *Proteus spp*. (2.5%). This result is almost the same as the research on Bangladeshi ready-to-eat food (Akter et al., 2019) but differs from the research result in China, which is only 17.2% (Ou et al., 2020). The prevalence of bacteria derived from meat may vary from country to country, type of meat sample, season of sampling, and isolation method.

In the study, the highest prevalence was *E. coli*, followed by *S. aureus, S Typhimurium, S. typhi*, and *Proteus spp*. However, if different types of fried chicken meat were found, 26 (88.9%) samples were positive for *S. aureus*, 26 (100%) samples were positive for *E. coli*. 7(30.4%) samples were positive for *S. Typhimurium*, 1 (4.3) sample were positive for *S. typhi*, and 1 (4.3%) samples were positive for *Proteus spp*. Of the 26 samples of fried chicken wings, 25 (96.3%) samples were positive for *S. aureus*, 26 samples (100%) were positive for *E. coli*, 5 (21.7%) were positive for *S.* Typhimurium, three samples (11.1%)) positive *S. typhi*. Of the 27 fried chicken breast samples, 26 (96.7%) were positive for *S. aureus*, 26 (98.8%) were positive for E. coli, 18 (33.3%) were positive for *S. Typhimurium*, 3 (11.1) %) positive for *S. Typhi* and 1 (4.2%) positive for *Proteus spp*.

Contamination of pathogenic *E. coli* along the broiler meat supply chain is a serious public health problem. This study found that 98.8% of ready-to-eat fried chicken samples were contaminated with *E. coli*. This result is different from several other studies that used samples of raw chicken meat or swabs directly from the cloaca of live broiler chickens (Hardiati et al., 2021; Indrawati et al., 2021; Rahayuningtyas et al., 2020; Salisu et al., 2020). Although most strains of *E. coli* are harmless and are normal flora in the intestines of humans and warm-blooded animals, some strains can cause severe foodborne illness in humans (Hui Zhang et al., 2017).

The average presence of *S. aureus* in fried chicken was (94.9%), different from that in Benin, which was only 9.9% (OP et al., 2016). Staphylococci are present in air, dust, waste, food, or food utensils, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. *Staphylococci* are also present in the nasal passages and throat and the hair and skin of 50% or more of healthy people. Although food handlers are the primary source of food contamination in food poisoning outbreaks, utensils, and environmental surfaces can also be a source of *S. aureus* contamination (Hyeon et al., 2013).

Staphylococcus aureus can cause food poisoning if the strains present in meat can produce enterotoxins. In addition, it has been hypothesized that handling or consumption of poultry meat may lead to colonization of the skin and mucosa (e.g., nasal and oral mucosa), which are considered important risk factors for *S. aureus* infection. Foodborne transmission of livestock-associated *methicillin-resistant S. aureus* (MRSA) is of particular concern given the recent emergence of this multi-drug-resistant bacterium in meat products, including poultry (Bortolaia et al., 2016). Chicken meat and its processed products contaminated with pathogenic bacteria will affect its quality and processed products. If consumed by humans, this chicken meat will cause foodborne diseases with mild to severe symptoms (Zelpina et al., 2020).

In this study, the prevalence of *Salmonella spp*. in ready-to-eat fried chicken is 17.45%, contrary to research in Bogor on processed chicken meat products at 38.09% (Novera et al., 2020), in Dhaka on chicken meat 8.62% (Siddiky et al., 2021). *Salmonella* is a crucial foodborne infection in humans worldwide and has signs of significant morbidity, mortality, and economic loss (Abate & Assefa, 2021). *Salmonella* is one of the most common foodborne pathogens, causing global foodborne disease outbreaks (Legese et al., 2020). Poultry, in particular, has been

considered the single leading cause of human salmonellosis, although the pathogen has been associated with a variety of food sources. Avian salmonellosis not only attacks the poultry industry but can also infect humans and is caused by the consumption of contaminated poultry meat and eggs (Gutema et al., 2021). Livestock products and by-products, especially eggs and poultry meat, are common carriers of Salmonella infection (Antunes et al., 2016). Typhoid and enteritis can be caused by foodborne *Salmonella* infection, which can be more severe in immunocompromised patients (Bintsis, 2017).

Recently, fast food consumption has increased widely, and the fast-food sector is becoming an important industry. Due to improper handling and management, such foods can quickly become contaminated with pathogenic microorganisms, which often cause foodborne illness and even death (Akter et al., 2019). Previous research revealed a high risk of infection associated with fast food consumption (Al et al., 2020).

CONCLUSION

This study shows that fast-food fried chicken sold in the Jember University campus is contaminated with pathogenic bacteria, namely *E. coli, S. aureus, S. typhimurium, S. typhi*, and *Proteus spp*. Therefore, it is necessary to ensure a food safety training program for all food vendors to reduce the risk of infection and provide safe food. Consumers should also be selective in choosing food and encourage fast-food fried chicken sellers to follow food safety rules and regulations. There needs to be regular monitoring by the ministry of health, with strict enforcement of the law, to improve the hygienic practices of fast-food vendors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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