

CONTAMINATION LEVEL AND PREVALENCE OF FOODBORNE PATHOGEN *Enterobacteriaceae* IN BROILER AND BACKYARD CHICKEN MEATS SOLD AT TRADITIONAL MARKETS IN SURABAYA, INDONESIA

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Accepted 21 May 2019, Published online 30 June 2019

ABSTRACT

This study aimed to determine contamination level and prevalence of foodborne pathogen *Enterobacteriaceae* in broiler and backyard chicken meats sold at traditional markets in Surabaya Indonesia. The average of *Enterobacteriaceae* count of backyard chicken meats ($7.61 \log_{10} \text{ cfu} / \text{g} \pm 0.62$) were significantly higher ($P < 0.05$) than those obtained from broiler chicken meats ($6.14 \log_{10} \text{ cfu} / \text{g} \pm 0.81$). Overall, the prevalence of *Enterobacteriaceae* in backyard chicken meats was significantly higher ($P < 0.05$) than broiler chicken meats, *Salmonella* spp. was the most common isolate recovered from backyard (96.67%) and broiler (81.67%) chicken meats, *E. coli* (backyard 76.67%; broiler 66.67%), *Citrobacter* spp., *Proteus* spp., *Yersinia* spp., *Klebsiella* spp., *Shigella* spp., *Enterobacter* spp., *Serratia* spp., *Edwardsiella* spp. and *Morganella* spp. *Morganella* spp. was found only in backyard chicken meats, and not found (0.00%) in broiler chicken meats. The high level of contamination and the prevalence of *Enterobacteriaceae* in chicken meats are related to poor sanitation and hygiene conditions in the traditional markets of Surabaya, Indonesia.

Key words: Contamination level, prevalence, *Enterobacteriaceae*, chicken meat, traditional markets in Surabaya

INTRODUCTION

Food borne disease remains a real and formidable problem on public health and economic losses in both developed and developing countries worldwide, including Indonesia. The Centers Disease Control (CDC) estimates that each year, roughly 48 million people get sick from a foodborne illness, in which 128,000 were hospitalized and 3,000 were

died (CDC, 2018). Several studies have shown that consumption of poultry meat is associated with the outbreak of foodborne illness (Painter *et al.*, 2013). Therefore, the safety guarantee of chicken meat products is an important issue to increase the consumption and production of poultry meat.

Chicken meat derived from broiler and backyard chickens was the most consumed meats as a source of animal protein by the Indonesian people compared to other meats because it is the cheapest and acceptable for all religions. Total consumption of

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chicken meat in Indonesia was 2.461.80 ton, in 2017 (Kementan Ditjen PKH, 2018). Surabaya is the second largest city in Indonesia, it has approximately 350.54 km² area (BPS of East Java Province, 2016). In 2017, total population and chicken meat consumption in Surabaya are 3.057.766 people and 19.77 ton, respectively (Kementan Ditjen PKH, 2018). Traditional markets in Surabaya are classified as a wet market, sell fresh chicken meats and the slaughtering is done manually inside the market. Chicken meats are sold at room temperature, in the open air. Manual slaughtering methods could increase the risk of chicken meat contamination with food borne pathogens from the intestinal contents due to the absence of standard procedures applied. The presence of high humidity in the wet market environment ideal for the colony formation of the food borne pathogen and the contamination of chicken meat has a negative impact on human health.

Chicken carcass as an ideal culture medium for the growth of many microorganisms where can be contaminated at several points of processing operation such as scalding, de-feathering and evisceration as well as cross-contamination from other carcasses, processing equipment and environment. According to WHO (1992), 25% of foodborne disease outbreaks are closely related to cross-contamination involving poor hygiene practices, contaminated equipment, contamination through food handlers, inadequate processing and storage. Some indicators such as the total of *Enterobacteriaceae* are used to evaluate enteric contamination and used in slaughterhouses as an indicator of faecal contamination (Barco *et al.*, 2014).

The *Enterobacteriaceae* is an important pathogen that is found in the intestinal tract and has caused many cases of foodborne illnesses around the world for many years. *Salmonella* spp., *E. coli*, *Proteus* spp. and *Klebsiella* spp. were the most dominant species of this family in all cases of food poisoning associated with several meat products, since they often contaminated raw and processed chicken meat products around the world (Al-Mutairi, 2011). *Enterobacteriaceae* includes several genera as primary causes of infection in the human digestive tract and several other genera as the main cause of opportunistic infections (including septicemia, pneumonia, meningitis and urinary tract infections) (Carroll *et al.*, 2015; Kumar, 2012). Previous research (Yulistiani *et al.*, 2017), showed that enteropathogenic bacteria found in chicken meat at traditional markets in Surabaya were resistant to some antibiotics. Thus, it is important to evaluate the emergence and spread of pathogenic bacteria derived from enteric contamination in chicken meat. The aim of this study was to determine the level of contamination and prevalence of foodborne patho-

gen *Enterobacteriaceae* in broiler and backyard chicken meats sold at the traditional market in Surabaya Indonesia.

MATERIALS AND METHODS

Sample collection

A total of 120 samples of raw chicken meats (broiler and backyard chicken meats, each of 60 samples) was obtained randomly from 12 traditional markets in Surabaya, Indonesia. Sample was packed in a sterile plastic bag and immediately transferred to the laboratory in the icebox for bacteriological testing. The name and location of the traditional market as a place of research sampling can be seen in Figure 1.

Microbial analysis

Determination of Enterobacteriaceae count

MacConkey agar (MCA, Oxoid) was used for the determination of *Enterobacteriaceae* counts (ISO, 2004; Morello *et al.*, 2002). Furthermore, total *Enterobacteriaceae* is expressed in average colony forming units per gram (mean cfu/g) and converted to log₁₀ based value.

Isolation of Enterobacteriaceae genera except for Salmonella spp.

Twenty-five gram of each sample was chopped aseptically and homogenized with 225 mL of Buffered Pepton Water (BPW, Oxoid). Subsequently, the pre-enrichment was incubated at 37°C for 24 hours. A loop full pre-enrichment broth was streaked on MacConkey agar plates (MCA, Oxoid) and incubated at 37°C for 24 hours. A single colony grew on the surface of Mac Conkey agar plate was observed with the specific criteria of *Enterobacteriaceae* genera (Roy *et al.*, 2012).

Isolation of Salmonella spp.

Salmonella spp. was detected according to the methods of BAM (2004) with some modifications. Twenty-five gram of each sample was chopped aseptically and homogenized with 225 mL of Buffered Pepton Water (BPW, Oxoid). Subsequently, the pre-enrichment was incubated at 37°C for 24 hours. One ml of Buffered Pepton Water (BPW, Oxoid) aseptically added into 10 mL Selenite Cystine Broth (SCB, Oxoid), incubated at 37°C for 24 hours. A loop full of the selectively enriched suspension was streaked onto Xylose Lysine Deoxycholate (XLD, Oxoid) agar and incubated at 37°C for 24 hours. A single colony grew on the XLD agar was observed with the specific criteria of *Salmonella* spp. (Roy *et al.*, 2012).



Fig. 1. Map of Surabaya traditional market location as a place of research sampling. This location indicated by the name of the traditional markets in this area (The insert is a map of Indonesia and the position of Surabaya in this map).

Identification of *Enterobacteriaceae* isolates

The criteria for confirmation of *Enterobacteriaceae* isolates were based on microscopic examination with Gram staining and biochemical profiling. The colonies with gram-negative and short-rod were evaluated by biochemical tests (IMViC test). The TSIA (Triple Sugar Iron Agar) test was conducted to check for their ability to ferment glucose, lactose and sucrose sugars, gas and H₂S production. The IMViC tests also for Indole production, Methyl Red, Voges-Proskauer, and the use of Simmon's citrate utilization (Baron *et al.*, 1994; Morello *et al.*, 2002; Kumar, 2012). The *Enterobacteriaceae* isolates were stored at -80°C in Luria-Bertani (LB, Difco) broth with the addition of 30% glycerol (v/v).

Data analysis and interpretation

The *Enterobacteriaceae* counts were transformed to log form before analysis. The data are expressed in absolute value and in percentages using the Microsoft Office Excel 2010 software. The level of contamination and prevalence of *Enterobacteriaceae* in chicken meat samples were compared between broiler and backyard chicken meats by Chi-square test at $P < 0.05$ (two-tailed),

with Yates' correction using Statistical Software Package for Social Sciences (SPSS, version 23, 2015). Statistical significance is defined in $P < 0.05$ at a confidence level of 5%.

RESULTS

Enterobacteriaceae counts in broiler and chicken meat

Table 1 shows that the average of the *Enterobacteriaceae* counts in all chicken meat samples was high. The average of the *Enterobacteriaceae* counts in backyard chicken meats (7.61 log₁₀ cfu/g ± 0.62) was significantly higher ($P < 0.05$) than broiler chicken meats (6.14 log₁₀ cfu /g ± 0.81) (Table 1).

Biochemical characterization of *Enterobacteriaceae* isolates

Identification of *Enterobacteriaceae* at genus level of 120 chicken meat samples, obtained eleven genera, *Salmonella* spp., *E. coli*, *Citrobacter* spp., *Proteus* spp., *Yersinia* spp., *Klebsiella* spp., *Shigella* spp., *Serratia* spp., *Enterobacter* spp., *Edwardsiella* spp. and *Morganella* spp. based on criteria for microscopic characteristics (short rod shape, gram-

Table 1. Contamination level of *Enterobacteriaceae* in broiler and backyard chicken meats

Type of chicken meat	No. sample	<i>Enterobacteriaceae</i> counts (Log ₁₀ cfu/g)		
		Min	Max	Mean ± SD
Broiler chicken meat	60	4.40	7.82	6.14 ± 0.81
Backyard chicken meat	60	5.72	8.72	7.61 ± 0.62
Total sample	120			
P-value		< 0.05		

Table 2. Biochemical characterization of *Enterobacteriaceae* isolates

<i>Enterobacteriaceae</i> Genera	Triple Sugar Iron agar test	Biochemical test				
		Motility	Indole production test	Methyl-red	Voges-Proskauer test	Simmon's citrate test
<i>Salmonella</i> spp.	K/A, or K/A gas + or K/A, H ₂ S or K/A gas, H ₂ S	+	-	+	-	±
<i>Escherichia coli</i>	A/A, or A/A gas + or K/A, or K/A gas +	+	+	+	-	-
<i>Proteus</i> spp.	A/A, H ₂ S or K/A, H ₂ S	+	±	±	+	±
<i>Citrobacter</i> spp.	A/A gas + or A/A, H ₂ S	+	±	+	-	+
<i>Shigella</i> spp.	K/A	-	±	+	-	-
<i>Yersinia</i> spp.	A/A, or K/A or A/A gas +	-	-	±	+	-
<i>Klebsiella</i> spp.	A/A gas +	-	±	-	+	+
<i>Edwardsiella</i> spp.	K/A, H ₂ S or K/A gas, H ₂ S	+	+	+	-	-
<i>Serratia</i> spp.	K/A or K/A gas +	+	-	-	+	+
<i>Enterobacter</i> spp.	A/A gas + or K/A gas +	+	-	-	+	+
<i>Morganella</i> spp.	K/A	+	+	+	+	-

Notes: K, Alkali (red); A, Acid (yellow); +, positive; - negative; ±, positive/negative.

negative) and the results of the biochemical test (TSIA and IMViC) is positive for each of *Enterobacteriaceae* genera (Table 2).

The prevalence among genera of *Enterobacteriaceae* in broiler and backyard chicken meat

The statistical analysis showed that overall, the prevalence of *Enterobacteriaceae* in backyard chicken meats was significantly higher ($P < 0.05$) than broiler chicken meats. Figure 2 shows the prevalence of *Salmonella* spp., *E. coli*, *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp. and *Morganella* spp. were significantly higher ($P < 0.05$) in backyard chicken meats than in broiler chicken meats. On the other hand, the prevalence of *Shigella* spp., *Yersinia* spp. and *Edwardsiella* spp. were significantly higher ($P < 0.05$) in broiler chicken meats than in backyard chicken meats. Whereas *Proteus* spp. and *Serratia* spp. there is no significant difference between broiler and backyard chicken meats. *Morganella* spp. was only found in backyard chicken meats.

Salmonella spp. was the most common isolate recovered from both backyard chicken meats (96.67%) and broiler chicken meats (81.67%), followed by *E. coli* (backyard 76.67%; broiler 66.67%), *Citrobacter* spp. (backyard 61.67%; broiler 50%), *Proteus* spp. (backyard 48.33%; broiler 51.67%), *Yersinia* spp. (backyard 41.67%; broiler 48.33%), *Klebsiella* spp. (backyard 46.67%; broiler 30.00%), *Shigella* spp. (backyard 16.67%; broiler 35.00%), *Enterobacter* spp. (backyard 23.33%; broiler 6.67%), *Serratia* spp. (backyard 20.00%; broiler 18.83%), *Edwardsiella* spp. (backyard 10.00%; broiler 16.67%) and *Morganella* spp. (backyard 1.67%; broiler 0.00%).

DISCUSSION

Our study showed a high level of *Enterobacteriaceae* contamination in chicken meats (Table 1). Similar results were reported by Rindhe *et al.* (2008) (6.27 log cfu / g) and Bhandari *et al.* (2013) (8.5

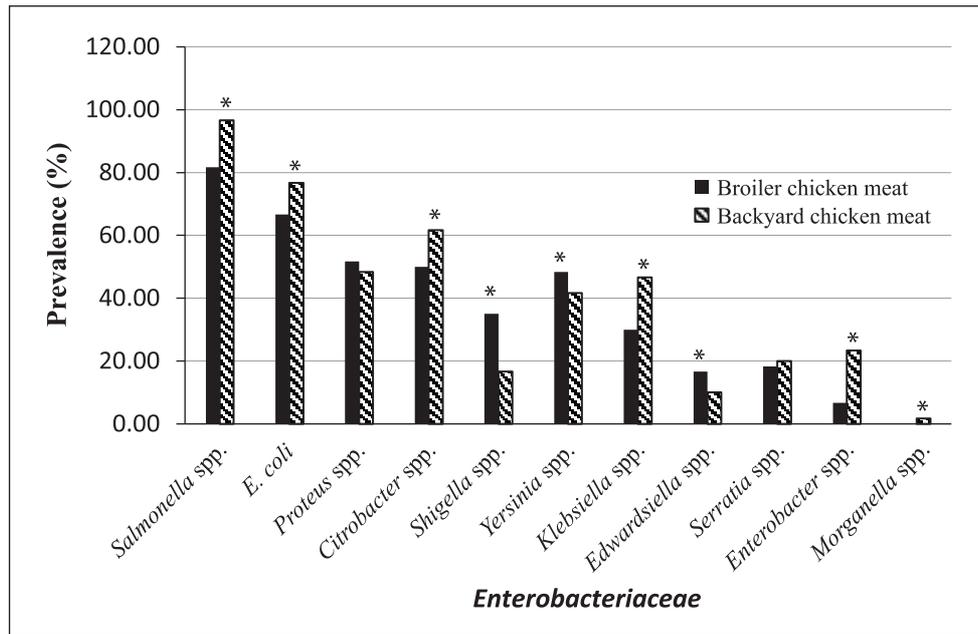


Fig. 2. The prevalence among genera of *Enterobacteriaceae* in broiler and backyard chicken meats. The symbols represent broiler chicken meat (■); backyard chicken meat (▨). *P values of < 0.05, Chi-squared test were considered significantly different.

log cfu/g). According to Mercuri and Cox (1979), the high *Enterobacteriaceae* counts in chicken meat indicated that the source of contamination came from the intestinal contents at the time of slaughtering, thus it can be used as the reason of enteric contamination. Evisceration is a step that, if carried out badly, lead to intestinal rupture and discharge of intestinal contents, and caused *Enterobacteriaceae* contamination in the chicken carcass (Baylis *et al.*, 2011). The high *Enterobacteriaceae* count in chicken meats also indicated poor sanitation conditions during slaughtering, handling, and preparation, as reported by Mulder and Krol (1976).

The low number of total and type contamination in broiler chicken meat compared to backyard chicken meat sold in traditional market Surabaya, is a result of the use of Antibiotic Growth Promoters (AGPs). The use of AGPs in broiler chicken can inhibit the growth and decrease the colonization of *Enterobacteriaceae* in the digestive tract (Pan & Yu, 2014; Mellen *et al.*, 2014), change the diversity and structure of microbial communities in chicken intestines (Lin, 2011), thus reducing the risk of chicken meat contamination. AGPs also causes antibiotic residues in chicken meat which can inhibit the growth of contaminant bacteria in chicken meat (Muaz *et al.*, 2018). Some studies reported the use of antibiotics as a feed additive in Indonesia causes antibiotic residues in broiler chicken meat (Werdiningsih *et al.*, 2013; Widiastuti & Anastasia, 2015; Saniwanti *et al.*, 2015). Our

result is similar to previous studies, which reported that on organic chicken with more restricted antimicrobial use, the average of *Enterobacteriaceae* count of organic chicken meats ($3.81 \log \text{ cfu/g} \pm 1.10$) were significantly higher ($P < 0.0001$) than those obtained from the conventional chicken ($2.66 \log \text{ cfu/g} \pm 1.30$) or conventional turkey meat ($1.44 \log \text{ cfu/g} \pm 0.661$) where they are greater use of antibiotic growth promoter (AGP) (Miranda *et al.*, 2008).

Eleven genera of *Enterobacteriaceae*, *Salmonella* spp., *E. coli*, *Citrobacter* spp., *Proteus* spp., *Yersinia* spp., *Klebsiella* spp., *Shigella* spp., *Serratia* spp., *Enterobacter* spp., *Edwardsiella* spp. and *Morganella* spp. were identified in the 120 chicken meat samples. Similar results have been reported by Gwida *et al.* (2014), which has detected several genera of *Enterobacteriaceae* such as *Proteus* spp., *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp. in raw chicken meat. The previous study by Shoaib *et al.* (2016) also showed the presence of *Escherichia coli*, *Salmonella*, *Klebsiella* spp., *Proteus* spp. and *Enterobacter* spp. in the liver organ of commercial broilers and poultry. While, the research by Noori and Alwan (2016) also showed the presence of *Escherichia coli*, *Salmonella*, *Citrobacter* spp. and *Proteus* spp. in broiler meats.

Enterobacteriaceae is an important family of gram-negative bacteria, which did not form spores, generally presented in the digestive tract, has 48 genera and 219 species, played an important role in enteric diseases and competed with other

pathogens (Baylis *et al.*, 2011). Ehara *et al.* (2000) stated that *Enterobacteriaceae* is a pathogenic bacterium and frequently isolated from gastroenteritis cases in humans, which causes gastrointestinal disorders ranging from mild diarrhea to mesenteric lymphadenitis. *Escherichia coli* is a member of *Enterobacteriaceae* which could act as commensal and pathogenic organisms, regarded as the cause of morbidity and mortality in the world (Miskinyte *et al.*, 2013). Pathogenic *E. coli* strains caused watery to bloody diarrhea, infantile diarrhea, traveler's diarrhea, hemorrhagic colitis, a hemolytic uremic syndrome in humans (Nataro & Kaper, 1998; Carroll *et al.*, 2016). *Salmonella* is a zoonotic bacteria that was transmitted to humans and caused two different types of diseases: typhoid fever and non-typhoid salmonellosis (Baylis *et al.*, 2011). Estimated that *Salmonella* caused more than 1 million foodborne illness every year and was responsible for more hospitalizations and deaths than any other type of bacteria or virus found in food. Food was estimated to be the source of 90 percent of *Salmonella* infections, whereas contaminated poultry was believed to be the primary source (FSIS, 2013). *Shigella* spp., *Yersinia*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Citrobacter* spp., *Serratia* spp., and *Edwardsiella* spp. were members of the *Enterobacteriaceae* that could cause diarrhea/dysentery, pneumonia, bladder infections, meningitis and nosocomial infections in humans (Baylis *et al.*, 2011; Kumar, 2012; Carroll *et al.*, 2016).

High prevalence of *Salmonella* in chicken meat, not only indicating poor sanitation conditions during slaughtering and marketing but also indicates the health status of poultry as the carrier of *Salmonella* (Paiao *et al.*, 2013). Poultry is the most important reservoir of *Salmonella* (Shah & Korejo, 2012). Antunes *et al.* (2003), also argued that the source of food poisoning in meat products, especially poultry meat, was *Salmonella*. *Salmonella* is one of the microorganisms which most frequently associated with outbreaks of diseases transmitted by food. The high prevalence of *Salmonella* spp. in chicken meat samples were also reported by Abdellah *et al.* (2009) (57%), Thanh Huong *et al.* (2009) (62.79%), Lertworapreecha *et al.* (2012) (67.5%) and Bhandari *et al.* (2013) (46.2%). In addition, Joshi & Joshi (2010) reported the prevalence of *Salmonella* which was isolated from all samples of chicken carcasses (100%).

The highest prevalence of *Salmonella* spp. in broiler and backyard chicken meats compared to other genera indicated that there was a lot of cross-contamination of *Salmonella*, due to poor sanitation and hygiene conditions and high humidity in the traditional market environment in Surabaya. The presence of *Salmonella* in the chicken intestines,

feathers, feet with poor sanitary and hygiene conditions could lead to cross-contamination and colonization of *Salmonella* in several places of the processing plant in the traditional market. *Salmonella* could be isolated from poultry processing equipment, especially in slaughtering and evisceration areas (Helke & Wong, 1994, Joseph *et al.*, 2001). The results are in accordance with Nidaullah *et al.* (2017), which reported a high prevalence of *Salmonella* (88.46%) in the chicken carcass, contact equipment used in poultry processing and environmental samples obtained from wet markets and small-scale processing units in Malaysia. Similarly, Modarressi and Thong (2010) reported a high prevalence of *Salmonella* (72.7%) in chicken meat samples in Kuala Lumpur. In South Africa, Van Nierop *et al.* (2005) reported that 60.6% of chicken carcass samples were contaminated by *Salmonella* spp. The high prevalence of *Salmonella* spp. in this result indicated that broiler and backyard chicken meats are potential reservoirs of *Salmonella* spp.

Poor sanitary condition and high humidity environment at slaughtering place and selling area of chicken meat in the traditional market are ideal conditions for the formation of *Salmonella* biofilms. This biofilm could last for a long time and tend to protect *Salmonella* from sanitizers, so it is very possible to cross-contamination of *Salmonella* in chicken meat. According to Joseph *et al.* (2001); Chmielewski and Frank (2003), microorganisms on wet surfaces have the ability to aggregate, grow into microcolonies, and produce biofilm. Growth of biofilms in food processing environments lead to increased opportunity for microbial contamination of the processed product. Microorganisms which formed biofilms are protected from sanitizers, increased the ability to survive and contaminate other foods, reducing the shelf life of the foods and increasing the spread of disease.

Many studies have shown that cross-contamination of poultry meat could occur during processing. In the food processing plant and wet market, the source of contamination could be workers, equipment such as defeathering machines, scalding water, floors, drains work benches and knives. *Salmonella* sp. could also survive for a long time inside biofilms which are formed on the surface, and these biofilms tend to protect *Salmonella* from detergents and sanitizers (Chmielewski & Frank, 2003; Giaouris *et al.*, 2014; Nidaullah *et al.*, 2017). In addition, several studies (Helke & Wong, 1994; Jones & Bradshaw, 1997; Joseph *et al.*, 2001) showed that *Salmonella* could be attached and formed biofilms on surfaces in food processing plants, including plastics, cement and stainless steels. The high prevalence of *Enterobacteriaceae* in this research indicated that broiler and backyard

chicken meats sold at traditional markets in Surabaya are a potential reservoir of food borne pathogen *Enterobacteriaceae* that can be transmitted to humans and cause illness and even death.

CONCLUSION

In conclusion, the high levels of contamination and the prevalence of food borne pathogens *Enterobacteriaceae* in broiler and backyard chicken meats sold at traditional markets in Surabaya have been confirmed. All used equipment and poor sanitary conditions during slaughtering and sales of chicken meats could act as a source of contamination. Our results emphasize the immediate need to implement strict hygiene and sanitation standards to ensure the safety of chicken meat and to eliminate sources of contamination.

ACKNOWLEDGEMENTS

The research was financially supported by the Ministry of Research Technology and Higher Education of the Republic of Indonesia through the Doctoral Dissertation Research Program (contract no. 19 / UN.63.8 / LT-CONTRACT / IX / 2017.)

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