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Microbial plate count and detection of *Escherichia coli* in pork meat samples from stalls in a public wet market in Cebu, Philippines

Ryan S. Yandug III[1]
Daniel C. Ventura Jr. [1]
Rochelle Haidee D. Ybañez[2]
Adrian P. Ybañez [1, 2,3,4]

[1]Southwestern University, Villa Aznar, Urgello St, Philippines
[2]Biology and Environmental Studies Program, Sciences Cluster, University of the Philippines Cebu, Lahug, Philippines
[3]Gullas College of Medicine, University of the Visayas, Banilad, Philippines
[4]University of Southern Philippines Foundation, Salinas Drive, Lahug, Philippines

Abstract

Meat safety issue is among the public health concerns associated with microbial pathogens. Contaminated meat can cause food-borne illnesses that can lead to serious medical conditions. In the local wet market setting in the Philippines, several common unhygienic practices may be observed. In this study, 75 pork meat samples (3 batches of 25 each) from 25 stalls were taken from a representative public wet market in Cebu, Philippines. Collected samples were subjected to microbial aerobic plate counts and Escherichia coli detection methods. Profile of meat vendors was also recorded. Obtained samples were subjected to microbiological assaying, starting from the non-selective bacterial growing to the selective E. coli media, and finally to the confirmatory chemical analysis of isolated organism for definitive E. coli identification. Results showed that the collected samples had high microbial plate count 1.1 x 10^8 to >5.9 x 10^8 cfu/g) exceeded the current limit (1 x 10^6 cfu/g) set by the National Meat Inspection Services (NMIS) of the Philippines. A total of 41 (55%) samples were found positive with E. coli, but the samples had a most probable number (MPN) value (<0.3 to 15) lower than NMIS standard (500). Consumers must be made aware of the health risks in buying pork meat from public wet markets. It is recommended that meat purchased from public markets must be properly heated before consuming to ensure that microbial pathogens are killed to avoid food-borne illnesses.

Key Words: Aerobic plate count; Escherichia coli; pork; public wet market; Cebu, Philippines.

Introduction: Meat safety, considered a minimum requirement for a successful livestock and meat production (Verbeke and Viaene, 1999), is among the public health concerns of consumers. Most meat safety issues are associated with bacterial pathogens that can cause human disease (Hirsh et al., 2004; Sofos, 2008). Bacterial presence on meat cannot be seen at post-mortem inspection. The production of visually clean meat is an important starting point for meat safety, but visual inspection can only detect gross fecal and other visible contaminants. Thus, microbiological assays can be

important to evaluate objectively the status of freshness and safety of the meat (Gracey and Church, 1999).

In the Philippines, meat products like pork are sold in local or public wet markets. Pork has been implicated as a major source of food-borne illness (EFSA, 2008). While the government agency National Meat Inspection Services (NMIS) evaluates meat produces from slaughter houses to ensure health safety of consumers, occurrences of illegal meat or "hot meat" reaching the market place are occasionally reported. These products may be contaminated and are still sold in the market. In other countries, meat recalls are being practiced (Marsh et al., 2004), which is usually not the case in the Philippines. On the other hand, meat in the public wet market may not be well preserved or unhygienic as some products are hanged and/or openly displayed in which consumers can touch and insects can easily access. These practices predispose transmission of bacterial pathogens like *Escherichia coli* to the consuming public. Handling of raw products has been shown to be critical to meat safety (Mor-mur and Yuste, 2010).

To date, there are no published reports concerning the safety of pork from public wet markets in Cebu and the Philippines. Hence, this study endeavored to evaluate pork samples from stalls in a representative local public wet market in Cebu, Philippines, which reflects common practices in other public wet markets in the country. Specifically, it aimed to obtain the profile of local market vendors and pork samples, to know the colony forming unit (CFU) and the most probable number (MPN) of *E. coli* of the obtained pork samples, and to determine the proportion of samples positive for *E. coli*.

Methodology:

Pork meat samples, vendor profile and study area: A total of 75 pork samples (collected once a week at 25 samples per collection), weighing 250g each was collected from 25 stalls in a public wet market operated by a local government unit in Cebu between 7 AM and 10 AM. Each sample was carefully sliced using individual sterile surgical blades, placed in a sterile plastic container, and then stored in a container with ice (approximately 7-8 ºC) until further laboratory processing for microbial analysis. Profile of vendor was obtained using a survey form. Microbial testing was conducted at the Diagnostic Laboratory, Veterinary Teaching Hospital, Southwestern University, Cebu City, Philippines.

Microbial assay: Series of procedures to quantify the number of microbial contamination and identify the *E. coli* for the samples collected were employed. Non-selective media (Laurel Stryptic Soy broth, plate count agar, and buffered peptone water), selective media (Eosin-Methylene Blue and *E. coli* broth) and confirmatory reagents (Indole-Methyl Red-Voges Proskauer-Citrate or IMViC) to identify *E. coli* from the samples were utilized.

Briefly, serial dilutions were made from the meat sample suspension starting with 1x 10^{-1} g/mL up to 10^{-5} g/mL. Each dilution was subjected to microbial assay by performing plate count method and identification by presumptive test and selective differentiation, confirmatory, biochemical test and Gram staining for the most probable number of *E. coli*. Plate count method was done by adding 1 mL of each dilution to a prepared plate count agar in petri dishes and incubated. Colony forming units were counted after incubation.

An aliquot of each dilution was added to 3 Durham's tubes containing Laurel Stryptic Tryptone broth for a presumptive test for *E. coli*. The presence or absence of gas formation was evaluated after incubation. A tube with suspected gas formation was further tested using *E. coli* broth in

Durham's tube for evaluation of gas formation. If gas formation was observed, the broth mixture was streaked in Eosin Methylene Blue prepared agar in Petri dishes for isolation of colony. The presence of metallic green sheen indicated positive for *E. coli*. Isolation of 5 green metallic sheen colonies from each plate (per dilution) into prepared plate count agar slants and subsequent incubation were performed. Each isolated bacteria were biochemically tested using IMViC.

Positive Indole, Methyl Red, Voges-Proskauer and Simon Citrate test gives a purplish red ring, red, pink to crimson and bromthymol blue color results, respectively. Samples were evaluated positive for *E. coli* if the following results were obtained: Indole test positive/negative, Methyl red test positive, Voges-Prokauer test negative and Simon Citrate agar color change negative (++--/-+--). Gram staining was done to positive isolates to check the morphology of the bacteria.

Data processing and analysis: Results were recorded and tabulated. Using a table for quantifying most probable number, results were compared to obtain the equivalent values in the reference table from NMIS. For the aerobic plate count, total number of colonies was counted from each sample. The results were statistically treated with simple averages and percentages.

Results and Discussion: The majority of the vendors were in the business for 6 years or less. None of them attended formal training on proper meat handling, and none was following the pre-identified hygienic practices in meat handling. All water supplies were sourced locally (Table 1). Non-attendance to training or compliance to proper hygienic practices in meat handling by the local meat vendors may reflect a lack of support from the government to provide proper training and to enforce strict regulation for adherence to standard practices. For the meat, the majority was sourced from the backyard (32%) and large scale piggery (32%), and from pigs slaughtered in less than 24 hours (56%).

Regardless of which batch the sample was collected, all aerobic plate counts (range: 1.1×10^8 to $>5.9 \times 10^8$ cfu/g) exceeded the current limit set by NMIS which is 1×10^6 cfu/g (Table 2) for chilled, frozen, comminuted meat and offals. This finding may be an effect of poor hygiene and sanitation practices (Mboto et. al, 2012; Guntner and Hautzinger,2007), which were also observed as presented in Table 1. Moreover, it may also be caused by the longer length of displaying time without proper storage (Guntner and Hautzinger, 2007) and exposure to heat from the environment (Gregory, 2010). However, sources of contamination could be from multiple sites including direct contact with the consumer (Mboto et. al, 2012) and fecal contamination to the carcass skin during dressing (Mor-mur and Yuste, 2010). As aerobic plate counts are still non-specific, the results indicate that the meat samples may not be safe for human consumption as other pathogens, including *Salmonella* and *Clostridium* spp. (Sofos, 2008; Mor-mur and Yuste, 2010; EFSA, 2008), may be present in the sample.

In each batch, samples positive with *E. coli* were detected (Table 3). The presence of *E. coli* has been shown to be an indicator of fecal contamination to the samples through direct contact from meat handlers with unsanitary practices (Feng et al., 2002). It may also be caused by the possible unhygienic handling of the meats during slaughtering and processing or due to possible contamination from the skin, mouth or nose of the handlers which might be introduced directly into the meat (Mboto et al., 2012). Although further strain identification of *E. coli* was not performed, possible implications for risk of acquiring disease due to the bacterium like "Traveller's Disease" and for possible epidemics of collibacillosis cannot be ruled out (Feng et. al. , 2002).

The lowest *E. coli* MPN was observed to be <0.3 and while the highest was 15. Though there were 41 (55%) samples that were found positive (Table 4), the values were found lower compared to the limit (500 MPN) set by the NMIS. *E. coli* contamination may not be prevented since it is a normal microflora of the intestinal tract in human and animals, and exposure may occur during the process of evisceration and the slaughter process (Hirsh et al., 2004). The result also implies that since high aerobic plate counts were seen, contamination may be attributed to other bacteria that maybe potentially pathogenic, including *Staphylococcus aureus* and *Salmonella* spp.

The result of this study implies that the meats sourced from the local wet market can be potentially harmful to the general public. The microorganisms that can be found in high number may be potentially pathogenic, which can cause food poisoning. Regardless of the low *E. coli* count on the samples, the general public is advised to cook meat thoroughly to reduce the risk of food-borne diseases from other contaminants and possible highly pathogenic strain of *E. coli*. Also, public should be made aware of the meat contamination and the proper handling of meat, including proper cooking and avoidance of eating raw or half done meat. Market Vendors should also be informed about the findings on the meat they are selling, and should further improve their current practices or procedures on hygiene, sanitation or meat storage. City Veterinary Office and NMIS must be strict in the regulation of hygienic practices done by the pork meat vendors and implement condemnation and confiscation of unclean, old stock meat. National Meat Inspection Services must include the routine microbiological screening of the meat sold in any public market.

As consumer's perception of meat safety can be influenced by government's safety awareness, campaigns and health consciousness (Liana et al., 2010), the government must increase its efforts in educating the public on how to avoid food-borne illnesses and in implementing rules to maintain sanitary practices in the market meat trade. Meat must be stored in a cool place or contained because environmental temperatures can affect microbial burdens in the product (Gregory, 2010).

As *E. coli* in raw foods is a significant reservoir of resistance and virulence genes (Van et al., 2008), further studies on antibiotic resistance in pork meat maybe conducted to evaluate the local situation. Foods contaminated with antibiotic resistant bacteria could be a major public health threat because of the possible transfer of mobile genetic elements from genes encoding antibiotic resistance determinants to other bacteria of human clinical significance. As a normal flora of both humans and animals, *E. coli* is considered a candidate vehicle (Van den Bogaard and Stobberingh, 2000). On the other hand, several preservation technologies to inactivate food-borne microbial pathogens (Zhou et al., 2010) where applicable must also be explored.

This study reported the microbial plate counts and detected *E. coli* from the pork samples obtained from the representative public wet market in Cebu, Philippines. Results showed that the obtained samples may pose a potential public health concern. Hence, meat obtained from public wet markets in the area must be well cooked to kill probable microbial pathogens.

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Table 1 Profile of the local wet market vendors and sold meat

Indicators	Frequency (n=25)	Percentage (%)
Number of years in business		
Less than a year	3	12
1 to 3 years	9	36
3 to 6 years	1	4
6 years above	2	8
Prefer not to say	10	40
Formal training attended related to meat handling		
With	----	---
None	25	100
Hygienic meat handling		
Appropriate wearing of gloves		
Practiced	---	---
Not practiced	25	100
Appropriate wearing of clothing		
Practiced	---	---
Not practiced	25	100
Wearing of hair nets		
Practiced	---	---
Not practiced	25	100
Use of proper storage		
Practiced	---	---
Not practiced	25	100
Source of water		
Local water source	25	100
Other source	---	---
Source of Meat		
Backyard	8	32
Large Scale Piggery	8	32
Prefer not to say	9	36
Date of Slaughter of Pig Source		
Less than a day	14	56
1-2 days	3	12
Prefer not to say	8	32

Table 2 Aerobic Plate Count (APC) of meat samples

Sample	Batch 1 EAPC/ml(g) (x 10^8)	Batch 2 EAPC/ml(g) (x 10^8)	Batch 3 EAPC/ml(g) (x 10^8)
1	>5.9	>5.9	1.09
2	>5.9	1.25	>5.9
3	>5.9	>5.9	1.32
4	1.3	>5.9	>5.9
5	>5.9	>5.9	>5.9
6	>5.9	1.51	>5.9
7	>5.9	>5.9	>5.9
8	>5.9	>5.9	>5.9
9	>5.9	>5.9	>5.9
10	1.1	>5.9	>5.9
11	>5.9	>5.9	>5.9
12	>5.9	>5.9	>5.9
13	>5.9	>5.9	>5.9
14	>5.9	>5.9	>5.9
15	>5.9	>5.9	>5.9
16	>5.9	>5.9	>5.9
17	>5.9	>5.9	>5.9
18	1.12	>5.9	1.48
19	>5.9	>5.9	>5.9
20	>5.9	>5.9	>5.9
21	>5.9	>5.9	>5.9
22	>5.9	>5.9	>5.9
23	>5.9	>5.9	>5.9
24	>5.9	>5.9	1.5
25	>5.9	1.14	>5.9
Total	**25**	**25**	**25**

Table 3 Number of samples positive for *E. coli*

Results	Batch			Total
	1	2	3	
Positive	9	19	13	41
Negative	16	6	12	34
Total	25	25	25	75

Table 4. Most Probable Number of *E. coli* from three batches of meat samples

Sample	Batch 1 MPN/g	Batch 2 MPN/g	Batch 3 MPN/g
1	<0.3	<0.3	<0.3
2	<0.3	3.0	<0.3
3	<0.3	7.2	3.0
4	<0.3	3.0	11
5	<0.3	6.1	3.0
6	<0.3	7.2	3.6
7	7.4	3.0	7.2
8	15	7.2	<0.3
9	<0.3	3.6	3.6
10	<0.3	<0.3	3.0
11	<0.3	<0.3	<0.3
12	<0.3	3.0	<0.3
13	<0.3	3.0	<0.3
14	3.0	3.6	3.0
15	3.0	14.0	3.0
16	<0.3	<0.3	3.6
17	3.0	<0.3	3.0
18	6.1	7.4	14
19	3.0	6.2	<0.3
20	3.6	<0.3	<0.3
21	<0.3	3.0	<0.3
22	<0.3	3.6	<0.3
23	3.6	3.0	<0.3
24	<0.3	3.0	<0.3
25	<0.3	15.0	<0.3
Total	**25**	**25**	**25**