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Prevalence of some food poisoning bacteria in local and imported retail pork by-products in Egyptian markets

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A very limited research work concerning foods of porcine origin in Egypt were obtained in spite of presence of a considerable swine population and consumers. This study was conducted to investigate the prevalence of food poisoning bacteria isolated from local and imported retail pork by-products in Egyptian markets. A total of 80 pork samples, including 60 local pork by-products and 20 imported ones were used. The isolated bacteria species after biochemical and serological typing were *Escherichia coli* (59) and distributed as *E. coli* O157(27), *E. coli* O146(18) and *E. coli* O111 (14) by 33.75, 22.5 and 17.5%, respectively followed by *Staphylococcus aureus* which was isolated from 23 (28.75%), *Salmonella* spp. was represented by *Salmonella typhimurium* (9) *Salmonella enteritidis* (7) and *Salmonella agona* (4), as 11.25,8.75, and 5%, respectively. Finally, *Listeria monocytogenes* was isolated from 9 samples as 11.25%. The bacterial isolates were sensitive to ciprofloxacin and more resistant to penicillin, gentamicin, amoxicillin and ceftazidime. The bacterial isolation is considerably more in the local pork by-products than the imported samples. On the whole, both types are commonly in permissible limits of the Egyptian food quality standard as the high A.P.C. were *Staphylococci* and *E. coli* followed by *Salmonella* spp., then *L. monocytogenes*. To the best of our knowledge, this is the first report on isolation and identification of food born bacteria from pork by-products in Egypt.

Key words: Pork by-products, local, imported, food poisoning bacteria, Egypt.

INTRODUCTION

Food-borne diseases are an important cause of morbidity and mortality worldwide. Food contamination with anti-biotic-resistant bacteria can be a major threat to public health, as the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections (Swartz, 2002). The prevalence of antimicrobial resis-tance among food-borne pathogens has increased during

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recent decades (Threlfall et al., 2000). Commonly, the developing countries have bad raw food hygiene, lack incidence of foodborne disease and antimicrobial resistance epidemiology, thus, management of biological hazards transmitted to humans by food consumption is of major health significance (Thi Thu et al., 2007). Good manufacturing/production practices and various interventions by slaughter and meat processing facilities play a large role in enhancing the safety of meat products. Baseline studies to determine microbial levels of pathogen prevalence can be used to assess the effectiveness of these programs and interventions (Bohaychuk et al., 2011).

Foods of porcine origin are an important vehicles associated with illnesses caused by foodborne pathogens, which lead to the development of antibiotic-resistant pathogens such as *Salmonella* spp., *Escherichia coli*, *Yersinia* spp., Staphylococci, *Listeria monocytogenes* (Wang et al., 2013).

Salmonella species are considered to be among the most important foodborne pathogens in the world and salmonellosis is still one of the most widespread foodborne bacterial illnesses in humans, with clinical manifestations ranging from asymptomatic state to severe disease (Galanis et al., 2006). The majority of infections are associated with the ingestion of contaminated foods such as pork. *Salmonella* in pork carcasses is a result of faecal contamination during slaughtering and processing. In this case, the carrier swine are the main initial source of contamination (Sylvia et al., 2005).

Foods of porcine origin are considered one of the sources of *E. coli* illnesses in humans. Most outbreaks of *E. coli* have been linked with the consumption of undercooked pork by-products; pork sausages and salami (Dias et al., 2013). The ability of *E. coli* to adapt to acid environments has caused this microorganism to be regarded as one of the most dangerous pathogens in fermented pork products. Several studies have shown that *E. coli* is able to survive the processes of fermentation, drying and storage when this microorganism is present in high numbers (Trotz-Williams et al., 2012).

L. monocytogenes poses a serious threat to public health, and the majority of cases of human listeriosis are associated with contaminated food. Pork meat and processed pork products were the sources of outbreaks of listeriosis during the last decade (Thévenot et al., 2006).

In Egypt, a very limited research work concerning epidemiological studies has implicated foods of porcine origin as an important vehicle associated with illnesses caused by foodborne pathogens, which lead to the development of public health hazards.

The present study was undertaken to provide a baseline data for strains isolated from local and imported pork and pork by-products in Egyptian markets.

METHODOLOGY

This study was conducted on 80 pork samples, including 6 types of local pork and pork by-products (n = 60) and 5 types of imported pork by-products (n = 20), purchased from pork retail markets. Samples were double-bagged at the source, refrigerated until delivery to the laboratory and then handled in such a manner as to prevent cross-contamination, and were examined within 1 day of purchase; they were chopped into small pieces, and 25 g from each sample was transferred to 225 ml of 1% buffered peptone-water and incubated for 24 h at 25 or 37°C.

Cultures were diluted to 10^{-4} in 0.1% peptone-water, and 100 µl volumes of different dilutions were spread on different specific agar media. The plates were then incubated at 37°C for 48 h, after which colonies were enumerated and the total bacterial (colony forming units) were calculated, as described by Azza et al. (2014).

Total plate count at 35°C (mesophiles)

The pouring technique recommended by AOAC (2000) was applied. 1 ml of each dilution was separately pipetted in sterile Petri-dishes. 15 ml of melted standard plate count agar (SPCA;Oxiod;CM325) at 42-45°C were poured; thoroughly mixed and then left to solidify. The inoculated plates were incubated at 35°C for 48 ± 2 h. The average number of colonies was determined and the aerobic plate count per gram was calculated as follows:

Mesophilic plate count/g/org. = No. of colonies × dilution.

Total plate count at 25°C (psychrotrophic bacteria)

The same technique of the pouring method was done as previously mentioned in mesophiles but the inoculated plates were incubated at 25°C for 48 h. The number of colonies/g was calculated in countable plates as follows:

Psychrotrophic count/g/org. = No. of colonies × dilution

Isolation and identification

The remaining TSB in the containers was incubated at 37°C for 12 h. Thereafter, the broth cultures were plated on selective and/or differential media, namely blood agar, MacConkey agar, Eosin methlene blue (EMB), xylose lysine desoxycholate agar, *Salmonella Shigella* agar (S.S. agar) mannitol salt agar and PALCAM agar. The plates were incubated at 37°C 24 h. Bacterial colonies in each medium were then characterized on the basis of colonial, cellular morphology and staining characteristics. On this basis, the colonies were categorized as Gram positive, catalase positive cocci; Gram positive short rods and Gram negative bacilli according to Koneman et al. (1983).

Biochemical identification

Organisms in each category were then identified, when possible, on the basis of biochemical characteristics by applying catalase activity test, IMVC reactions tests, hydrogen sulfide production (triple sugar iron agar, TSI), hydrolysis of urea, sugar fermentation, nitrate reduction and detection of motility according to Carter and Cole (1990).

Serological identification

The somatic (O) antigen of *E. coli* was determined by slide agglutination test according to Edwards and Ewing (1972), while

Flagellar (H) antigen sereotyping was carried out according to Davies and Wray (1997). Anti-O-sera were obtained from DENKA SEIKEN CO LTD Tokyo, Japan. *Salmonella* spp. was serotyped according to Bale et al. (2007). *Listeria* spp. was serologically identified with factor sera according to Schnberg et al. (1989).

Sensitivity test for antibiotics

It was carried out according to the National Committee for Clinical Laboratory Standards, 2000.

Preparation of standard suspension

Some of typical colonies of each isolate were suspended in Mueller-Hinton broth and incubated at 37°C for 8 h till its turbidity exceeds the turbidity of standard McFarland tube No. 0.5.

Inoculation of the test plates

A sterile cotton swab was dipped into standardized bacterial suspension. The swab was then used to streak the dried surface of Mueller-Hinton agar plate in three different planes by rotating the plates to be sure for even distribution of the inoculums.

Placement of the discs

The antimicrobial discs were placed on the inoculated place using gentle pressure by sterile pointed forceps on the agar to ensure complete contact with the surface. Then the plates were incubated at 37°C for 24 h.

Reading of the results

The degree of sensitivity was estimated by measuring the visible clear zone of inhibition produced by the diffusion of the used antimicrobial disc into the surrounding medium. Interpretation of the results was done according to the National Committee for Clinical Laboratory Standards (2000).

RESULTS AND DISCUSSION

From the results presented in the Tables 1 and 2, the bacterial isolation is considerably more in the local pork and pork by-products than the imported samples. On the whole, both types are commonly in permissible limits of the Egyptian food quality standard as the high T.P.C. were *Staphylococci* and *E. coli* followed by *Salmonella* spp. then *L. monocytogenes*. Manguiat and Fang (2013) reported high levels of aerobic plate count, *E. coli* and *S. aureus*. The highest counts obtained were 8.2, 5.4, 4.4 log and 3.9 log cfu g^{-1}, respectively, *Salmonella* was found in 8% of the samples.

Table 3 shows the bacteria species isolates after biochemical and, serological typing were *E. coli* (59), and distributed as *E. coli* O157(27), *E. coli* O146 (18) and *E. coli* O111(14) by 33.75,22.5 and 17.5%, respectively.

Shiga toxin-producing *E. coli* (STEC) strains are food-borne pathogens that are an important public health concern. STEC infection is associated with severe clinical diseases in human beings, including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), which can lead to kidney failure and death. Tseng et al. (2014) stated that a number of STEC outbreaks and HUS cases have been attributed to pork products in spite of the role that swine play in STEC transmission to people and the contribution to human disease frequency requires further evaluation.

Magwedere et al. (2013) investigated STEC O-groups, responsible for the majority of *E. coli* infections in human beings, in retail pork meat (n = 16), and represented 8 samples (9%). Johnson et al. (2005) concluded that ground pork may be an important vehicle for community-wide dissemination of *E. coli* and Rode et al. (2012) mentioned that dry-fermented sausages are considered possible risk pork products regarding STEC.

S. aureus was isolated from 23 as 28.75%, and this result was nearly similar to the results obtained by Koláčková et al. (2014) who evaluate the contamination of raw pork meat with *S. aureus* in the retail market and found that 21.8% were found to be positive, and Atanassova et al. (2001) who detected *S. aureus* as 25.9%. We observed that the isolation of *S. aureus* from salami and mortadella was the least and this may be attributed to their low pH and proved to be a difficult environment for the survival of *S. aureus* (Wallin-Carlquist et al., 2010).

The isolated *Salmonella* spp. were represented by Salmonella *typhimurium* (9) *Salmonella entertidis* (7) *and Salmonella agona* (4), as 11.25, 8.75 and 5%, respectively. These results agreed with that of Kerouanton et al. (2013 who mentioned *S. typhimurium* as the most often isolated serotypes in pigs, pork and pork by-products, also Lin et al. (2014) isolated *S. enteritidis* from pork.

Our study shows that *L. monocytogenes* was isolated from nine samples as 11.25%, but in very low colony count and this agreed with the result obtained by Ristori et al. (2014) who mentioned that the *L. monocytogenes* populations were $<10^2$ cfu/g in the majority of samples.

Finally, the obtained results of the study revealed that the porcine liver and kidney are the highest bacterial colony populations among the samples followed by minced pork and these results are supported by those obtained by Sasaki et al. (2013) who suggested that the consuming swine livers and kidneys without proper heat treatment may increase the risk of foodborne illnesses.

As shown in Table 4, 100 and 30% of the *S. aureus* isolates were resistance to penicillin and amoxicillin respectively, while 91 and 83% were sensitive to ciprofloxacin and ceftazidime respectively. These results agree with Espinosa et al. (2011) who mentioned that the rate of ciprofloxacin and amoxicillin sensitivity for *S. aureus* is 100 and 60%, respectively. The isolates showed 100% resistance to penicillin.

As shown in Tables 5, 6 and 7, the *E. coli* isolates were more resistant to amoxicillin and ceftazidime, and more

Table 1. Enumeration of the isolated bacteria from retail local pork and pork by-products.

Sample	Microorganism							
	Staphylococci		*Escherichia coli*		*Salmonella spp.*		*Listeria* spp.	
	T.P.C.	No	T.P.C	No	T.P.C	No	T.P.C	No
Salami	$4x10^2$	2	$1x10^2$	8	2 x10	1	0	0
Mortadella	$4x10^2$	2	$1.2x10^2$	2	2 x10	1	0	0
Sausage	$3x10^3$	1	$3 x10^2$	1	$1x10^2$	2	10	2
Minced meat	$4 x10^3$	3	$3 x10^3$	0	$1x10^3$	2	14	2
Liver	$8 x10^3$	5	$3 x10^3$	3	$2x10^2$	2	18	2
Kidney	$6 x10^4$	3	$3 x10^3$	3	$3 x10^2$	3	22	3

Table 2. Enumeration of the isolated bacteria from retail imported pork by-products.

Sample	Microorganism							
	Staphylococci		*Escherichia coli*		*Salmonella spp.*		*Listeria* spp.	
	T.P.C.	No	T.P.C.	No	T.P.C.	No	T.P.C.	No
Salami	$1x10^2$	1	$1x10^2$	2	1x10	1	0	0
Mortadella	4 x10	1	1 x10	1	1 x10	1	0	0
Bavarian sausage	$3 x10^4$	3	$3 x10^2$	4	$3 x10^2$	4	8	1
Canadian bacon	$2 x10^2$	1	$2 x10^2$	3	2 x10	1	0	0
Smoked bacon	$3 x10^3$	1	$2 x10^2$	2	1 x10	1	0	0

Table 3. Identification of the isolated bacteria from retail local, imported pork by-products.

Microorganism	(n=80)
Staph.aureus	18.75%
E.coli O157	33.75%
E.coli O146	22.5%
E.coli O111	17.5%
Salmonella typhimurium	11.25%
Salmonella enteritidis	8.75%
Salmonella agona	5%
Listeria monocytogenes	11.25%

Table 4. Antibiogram sensitivity test of *S. aureus* isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	7	30%	3	13%	13	57%
Penicillin	23	100%	0	0%	0	0%
Ciprofloxacin	0	0%	2	8.5%	21	91%
Ceftazidime	2	8.5%	2	8.5%	19	83%

The percent was calculated according to the total number of *S. aureus* isolates (n=23).

sensetive to gentamycine and ciprofloxacin.These results greatly agree with that of Espinosa et al. (2011) who mentioned that *E. coli* isolates are resistant to amoxicillin (70%) and were sensitive to ciprofloxacin (100%).

As shown in Table 8, 9 and10, the *Salmonella* isolates were more resistant to amoxicillin, gentamycine and

Table 5. Antibiogram sensitivity test of *E. coli* O157 isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	20	74%	4	15%	3	11%
Ciprofloxacin	14	52%	3	11%	10	37%
Gentamicin	9	34%	2	7%	16	59%
Ceftazidime	19	70%	3	11%	5	19%

The percent was calculated according to the total number of *Escherichia coli* O157 isolates (n=27).

Table 6. Antibiogram sensitivity test of *E. coli* O146 isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	11	61%	3	17%	4	22%
Ciprofloxacin	8	45%	4	22%	6	33%
Gentamicin	2	11%	2	11%	14	78%
Ceftazidime	10	55.5%	1	5.5%	7	39%

The percent was calculated according to the total number of *Escherichia coli* O146 isolates (n=18).

Table 7. Antibiogram sensitivity test of *Escherichia coli* O111 isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	9	65%	3	21%	2	14%
Ciprofloxacin	6	43%	3	21%	5	36%
Gentamicin	0	0%	3	21%	11	79%
Ceftazidime	8	58%	4	28%	2	14%

The percent was calculated according to the total number of *Escherichia coli* O111 isolates (n=14).

Table 8. Antibiogram sensitivity test of *Salmonella typhimurium* isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	9	0%	0	0%	0	0%
Ciprofloxacin	2	22%	0	0%	7	78%
Gentamicin	8	89%	1	21%	0	0%
Ceftazidime	3	33%	1	11%	5	56%

The percent was calculated according to the total number of *Salmonella typhimurium* isolates (n=9).

Table 9. Antibiogram sensitivity test of *Salmonella enteritidis* isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	6	86%	1	14%	0	0%
Ciprofloxacin	3	43%	1	14%	3	43%
Gentamicin	5	72%	1	14%	1	14%
Ceftazidime	4	58%	2	28%	1	14%

The percent was calculated according to the total number of *Salmonella entertidis* isolates (n=7).

Table 10. Antibiogram sensitivity test of *Salmonella agona* isolates.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	4	100%	0	0%	0	0%
Ciprofloxacin	0	0%	0	0%	4	100%
Gentamicin	3	79%	1	21%	0	0%
Ceftazidime	4	100%	0	0%	0	0%

The percent was calculated according to the total number of *Salmonella agona* isolates (n=4).

Table 11. Antibiogram sensitivity test of *Listeria monocytogenes*.

Antibacterial agent	Resistant		Intermediate		Sensitive	
	No	%	No	%	No	%
Amoxicillin	1	11%	1	11%	7	78%
Penicillin	0	100%	1	11%	8	89%
Ciprofloxacin	5	55.5%	3	33.5%	1	11%
Ceftazidime	6	67%	1	11%	2	22%

The percent was calculated according to the total number of *Listeria monocytogenes* isolates (n=9).

ceftazidime, and more sensetive to ciprofloxacin. These results nearly agree with that of Espinosa et al. (2011) who mentioned that *Salmonella* isolates were100% resistant to amoxicillin and 100% sensitive to ciprofloxacin. Also, Dechen et al. (2011) and Sang et al. (2012) mentioned that *Salmonella* isolates were 100% sensitive to ciprofloxacin.

As shown in Table 11, the *L. monocytogenes* isolates were more resistant to ciprofloxacin and ceftazidime, and more sensetive to penicillin and amoxicillin and these findings agree with those of Moreno et al. (2014) who mentioned that isolates of *L. monocytogenes* were susceptible to penicillin and possessed at least intermediate resistance to fluoroquinolones.

Conclusion and recommendation

Data regarding the bacteriological count and isolation in APC, *S. aureus*, *E. coli*, *Salmonella* spp. and *L. monocytogenes* from local and imported pork and pork by-products were relatively lower than the Egyptian food quality standard. Regardless, samples were found to be satisfactory due to low levels of aerobic plate count, but the attention should be given to the antibiotic resistant isolates. Generally,the total bacterial counts were lower in processed, heat treated pork by-products than the raw and porcine organs, so, proper heating and processing of pork and pork by-products is recommended to minimize the public hazards. The obtained data seemed to be firstly described in Egyptian retailed pork by-products and need more investigations and studies.

Conflict of interests

The authors did not declare any conflict of interest.

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