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Detection of *Pseudomonas* spp. in Chicken and Fish Sold in Markets of Assiut City, Egypt

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Abstract

Background: *Pseudomonas* spp. are of important food spoiling bacteria. They present around the world and are isolated from diverse sources. The main objective of this survey was to detect *Pseudomonas* spp. in fresh chicken and fish obtained from markets of Assiut city, Egypt.

Methods: In this cross-sectional survey, 50 samples of fresh raw chicken and 50 samples of fresh raw fish meat were randomly collected from Assiut city markets, Egypt. Then, in laboratory, cultural and PCR techniques were carried out to detect *Pseudomonas* spp. in the samples.

Results: *Pseudomonas* spp. were isolated from 80% of the examined chicken samples and found in all the examined fish samples. All the isolates were confirmed as *Pseudomonas* spp. using *16S rRNA*-PCR. The average number of *Pseudomonas* spp. in chicken flesh and *Tilapia nilotica* were 2.6×10^4 and 2.8×10^3 CFU/g, respectively; while this rate was 1.4×10^5 CFU/g for African catfish (*Clarias gariepinus*).

Conclusions: This study indicated contamination of fresh raw fish and chicken samples with *Pseudomonas* spp. which may be attributed to cross contamination. This contamination predicts inadequate shelf life of the examined samples especially African catfish.

Introduction

Pseudomonas spp. present everywhere and are isolated from a multiplicity of sources including drinking water, domestic and wild animals, human beings, plants, and also from a variety of foods. These Gram-negative bacteria are non-fermentative rods, aerobic, oxidase-positive and motile with polar flagella (Adams and Moss, 2008; Arnaut-Rollier et al., 1999; Franzetti and Scarpellini, 2007; Jay et al., 2005).

Shelf life of food is known as the time length when the quality of food is still satisfactory under exact conditions of distribution, storage, and display. Spoilage is the procedure by which food is deteriorated and becomes unacceptable for humans or its quality is reduced making it inappropriate for sale or consumption (Jay et al., 2005). Some bacterial species firstly cause change in sugars by oxidation and produce a fluorescent pigment; others produce alkali. The psychrotrophic *Pseudomonas* strains are specific spoilage organisms (SSO) of meat, poultry and fish, detected by analyzing the nitrogenous components producing the volatile compounds (aldehydes, ketones and esters) responsible for the off-flavor produced at the point of spoilage (Alasalvar et al., 2011; Gracey et al., 1999; Yagoub, 2009). Food microbiologists are worried

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about the presence of these organisms and also about its number. *Pseudomonas* spp. may only represent the minority of the total microflora at the beginning, but become the dominant at the end of the shelf life. The quantity of present SSOs can be used to predict the residual shelf life of products causing identification of this organism as a main concern. The shelf life of any food product can be detected from beginning of storage period and ending when the SSOs reach the least spoilage level (Arnaut-Rollier et al., 1999; Bremner, 2002; Franzetti and Scarpellini, 2007; Roberts and Greenwood, 2003). *Pseudomonas* spp. decrease the shelf life of food products and so their quality by producing lipolytic and proteolytic enzymes which is the main cause of food spoilage during the storage (Franzetti and Scarpellini, 2007).

The main objective of this survey was to detect *Pseudomonas* spp. in fresh raw chicken and fish sold in markets of Assiut city, Egypt using cultural and biochemical tests as well as confirmation by PCR amplification of the target gene.

Materials and methods

Sampling

In this cross-sectional survey carried out during 2014, 50 samples of fresh raw chicken and 50 samples of fresh raw fish meat including *Tilapia nilotica* (n=25) and African catfish (*Clarias gariepinus*, n=25) were randomly collected from Assiut city markets, Egypt. The samples were kept in ice box during transportation till instant analysis in the laboratory.

Isolation and enumeration of Pseudomonas spp.

Skinned meat from chicken, and flesh from the dorsal half of the fish were used for the analysis. Twenty five g of the flesh of each chicken and fish sample were homogenized in 225 ml peptone water**,** and then, serial decimal dilutions were prepared. Amount of 0.1 ml of each dilution was spread on *Pseudomonas* cetrimide, nalidixic acid (CN) agar; *Pseudomonas* agar base contain 10 ml/l glycerol and selectivity made by inclusion of cetyltrimethyl ammonium bromide (cetrimide; 200 mg/l) and nalidixic acid sodium salt (15 mg/l) and were incubated at 25 °C for 48 h. All colonies that developed on the medium were counted and confirmed their identity as *Pseudomonas* by oxidase testing (Roberts and Greenwood, 2003).

DNA extraction

Overnight cultures on nutrient broth were collected in sterile microtubes and washed by phosphate buffer saline (PBS) triple. DNA extraction was performed by boiling and then centrifugation at 10000 rpm for 10 min; the supernatant was stored at -20 °C for next steps.

PCR analysis

Partial amplification of *16S rRNA* gene was performed using the primer pairs *Pseudomonas*-F (5'-CTACGGGAGGCAGCAGTGG-3') and *Pseudomonas*-R (5'-TCGG-TAACGTCAAAACAGCAAAGT-3'), producing an amplicon with 150 bp (Purohit et al., 2003). In 25 µl reactions containing 100 pmol of each primer, 1X GoTaq Green Master Mix (Promega, USA), and 100 ng of DNA template, amplification was performed. Cycling condition supplied by Techne Thermocycler (UK) was 95 °C for 5 min as for first denaturation and followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s and a final extension at 72 °C for 10 min. Positive and negative control were used in each round with *P. aeruginosa* ATCC 27853 and water, respectively. PCR amplicons were analyzed by 1% agarose gel electrophoresis, visualized under UV transillumination.

Results

Pseudomonas spp. were isolated from 80% of the examined chicken samples, but found in all the examined fish samples (Table 1) and finally confirmed as *Pseudomonas* spp. using *16SrRNA*-PCR (Fig. 1). The average number of *Pseudomonas* spp. in chicken flesh and *Tilapia nilotica* was 2.6×10^4 CFU/g and 2.8×10^3 CFU/g, respectively; while this rate was 1.4×10^5 CFU/g for African catfish (*Clarias gariepinus*).

Table 1: Total count of *Pseudomonas* spp. in examined raw chicken and fish samples

Samples	Samples size	No. of samples with *Pseudomonas* spp. (%)	Count of *Pseudomonas* spp.		
			Min	Max	Mean
Chicken	50	40 (80)	5×10	2×10^5	2.6×10^4
Tilapia nilotica	25	25 (100)	5×10^2	1×10^4	2.8×10^3
African catfish (*Clarias gariepinus*)	25	25 (100)	8×10^4	1.7×10^5	1.4×10^5

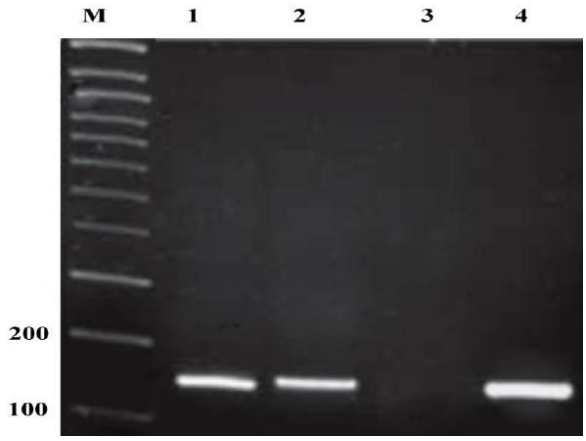

Fig. 1: PCR amplification results; Lane M: 50 bp DNA ladder; Lanes 1, 2: *Pseudomonas* positive strains; Lane 3: negative control; Lane 4: positive control with *Pseudomonas aeruginosa* ATCC 27853

Discussion

In this study, *Pseudomonas* spp. was found in 80% of chicken samples and 100% of fish samples. Enumeration of *Pseudomonas* spp. in the collected fish and chicken samples is of highly importance because this bacterium is often considered as an indicator of food quality. Also, some *Pseudomonas* spp. may cause food-borne disease (Begum et al., 2010; Jay et al., 2005). The sources of isolated *Pseudomonas* spp. are ubiquitous including marine and fresh water, animals, humans, plants (Adams and Moss, 2008; Alasalvar et al., 2011; Roberts and Greenwood, 2003). Scalding of poultry may destroy *Pseudomonas*, but the next processing steps re-contaminate the product. Many studies reported that there is a direct relation between the initial number of *Pseudomonas* and the shelf life of the product at refrigeration temperatures and spoilage occurs when the number of this organism range from 10^7 to 10^8 CFU/g. To achieve ideal shelf life and sensory demand, the initial loads of *Pseudomonas* spp. should be <100 CFU/g on meat products under aerobic conditions (Bremner, 2002; Mead, 2005).

Similar survey by Keskin and Ekmekci (2008) showed that average number of psychrophilic bacteria in chicken meat and sardine fish samples were 1.5×10^4 CFU/g and 5.3×10^4 CFU/g, respectively. Liu et al. (2010) stated that *Pseudomonas* spp. were SSOs in various fish species from temperate and tropical waters which resulted in clear off-odors and large amounts of total volatile basic nitrogen (TVBN). The organism produces huge amounts of proteolytic enzymes under aerobic refrigeration or ice storage and their presence could be used to predict the shelf life of fish (Jay et al., 2005). Khidhir et al. (2014) found that *Pseudomonas* spp. was 59.16×10^2 CFU/g for silver carp sold in Sulaimania markets, Iraq. Higher count was recorded by Begum et al. (2010) who collected fish samples from the super shop in Bangladesh, and reported that the highest count of *Pseudomonas* spp. was found in *Tilapia* (8.68×10^5 CFU/g). Arnaut-Rollier et al. (1999) in a study on fresh and refrigerated chicken skin, showed the predominance of four main *Pseudomonas* spp. including *P. fragi*, *P. lundensis*, *P. fluorescens* biovars and an unidentified strain similar to *P. fluorescens* biovars. In contrast, Iroha et al. (2011) could not isolate *P. aeroginosa* from any of 100 chicken meat samples and Hinton et al. (2007) found that although no psychrotrophs were recovered from broiler carcasses immediately after washing with chlorinated or EO water, however *Pseudomonas* spp. was the predominant psychrotrophs isolated from all carcasses refrigerated for 7 to 14 days. Yagoub (2009) was able to isolate *Pseudomonas* spp. at lower rate of 62% in the fishes collected randomly from markets of Khartoum, Sudan and stated that the isolation of *Pseudomonas* spp. from the samples is of highly significance because the organism is a potential pathogenic bacterium for human and as a pointer of food quality as a spoilage organism. Although the results of previous mentioned researches were somewhat diverse, but it seems that most of them indicated that *Pseudomonas* spp. is one of the most prevalent spoiling microflora in raw chicken and fish.

Conclusion

This study indicated contamination of fresh raw fish and chicken flesh with *Pseudomonas* spp. which may be attributed to cross contamination. This contamination predicts inadequate shelf life of the examined samples especially African catfish (*Clarias gariepinus*).

Conflicts of interest

No conflict of interest.

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