Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Food Hygiene

Prevalence and PCR Screening of *Pseudomonas* Isolated from Some Meat Products in Egypt

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Received: 19/7/2021 Accepted: 18/8/2021

ABSTRACT

A total of 200 random samples of meat products represented by minced meat, beef burger, beef kofta and beef sausage (50 of each) were collected from different supermarkets at El Menofiya and Cairo Governorates. The collected samples were examined for isolation of Psuedomonas and molecular characterization by using Multiplex PCR. psuedomonas was isolated from examined samples with the percentage of 18% (9) ,30% (15), 26% (13) and 22% (11) in minced meat, beef burger, beef kofta and beef sausage, respectively. The isolated pseudomonas could be serologically identified as *P. aeruginosa ,P. diminuta ,P. fluorescens , P. proteolytica, P. fragi , P. putrefaciens and P. putida.* Multiplex PCR results showed that the prevalence of virulence genes of *P. aeruginosa* were exotoxin A gene (ETA) 36%(4), gryase B gene (gyrB) with outer membrane protein gene (oprL) 54% (6), exotoxin A gene (ETA) with outer membrane protein gene (oprL) 18% (2) and gryase B gene (gryB) with exotoxin A gene (ETA) with outer membrane protein gene (oprL) (18%(2). The prevalence of Pseudomonas species in meat products samples is highly significant Therefore, its presence should be avoided during first stages of food processing.

Keywords: *Meat products, Psuedomonas, virulence genes*

INTRODUCTION

Meat products such as minced meat, beef burger, kofta and sausage are highly demanded and considered more attractive for consumers than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving. Inspite of the importance of meat products to consumers, they can be contaminated with several types of food borne microorganisms from different sources during handling, preparation and storage practices (Al-Mutairi., 2011).

The deterioration of meat depends on pH level, accessibility of oxygen, biodiversity of bacterial bunches, and capacity temperature (Ercolini *et al.,* 2010). These components,in turn, are closely related with the development of deterioration microbes.

The man handles of temperature control and destitute nourishment dealing with may energize the development of microorganisms which leads to defilement and deterioration of nourishment (Gour *et al.,* 2014). Storage temperature, however, is the most important factor that affects the growth of bacteria present in meat. Psychrotrophic bacteria can grow at refrigerated conditions, and temperature can affect various microbial growth parameters including maximum growth rate and total bacterial counts (Mataragas *et al.,* 2006). Pseudomonas spp. may be a major psychrotrophic bacterium that produces proteinase and its ideal pH is from 6.5 to 8.0. proteinase hydrolyses chicken protein and cause decay (Nowak *et al.,* 2012). Pseudomonas spp. found all over and

are disconnected from a distinctive of sources like drinking water, human creatures, plants, additionally from a difference of nourishments. Pseudomonas is a high-impact, Gram-negative bacterium that's commonly found in soil. It can develop well in a run of temperature levels, from 2 to 35 °C (Ercolini *et al.,* 2010), and can be effortlessly found in chilled nourishment items, as well as nourishment arranged at room temperature. Within the nourishment industry, different nourishments harbor exceptionally differing Pseudomonas species. Most of the segregates have the capacity to develop at room temperature and are competent of discharging chemicals that can influence the in general quality of the nourishment items counting cold-stored nourishment (Caldera *et al.,* 2016). Four species of Pseudomonas, namely, P. fluorescens, P. lundensis, P. fragi, and P. viridiflava, are the most cause of food deterioration since these life forms create enzymes and shape abiofilm, hence causing deterioration in refrigerated nourishment (Rawat 2018). For occurrence, P. fluorescens has been related with decay of chicken carcasses. When its populace comes to 108 cfu / ml, it may cause the generation of a solid foul scent (Wang *et al.,* 2014). In expansion, P. fragi is commonly known to ruin drain and meat (Ercolini *et al.,* 2010). This may too lead to the generation of odor and sludge in nourishment items. Reusing the fixings put away at room temperature for few hours posture hazard to customers particularly in case they are resistant compromised (Tsao *et al.,* 2018). Separated from being a deterioration microorganism, Pseudomonas spp. may cause urinary and blood stream disease. Typically, due to the truth that they create resistance to certain anti-microbials (Golemi-Kotra *et al.,* 2008). Pseudomonas species diminishes the capacity life of nourishment items and subsequently their quality by creating proteins as proteolytic and lipolytic which are the essential reason of nourishment decay amid capacity (Franzetti and Scarpellini, 2007). Therefore, the current study was carried out to evaluate the incidence of Pseudomonas species and molecular characterization of isolated strains from some meat products.

MATERIAL AND METHOD

Collection of samples:

A total of 200 samples of meat products represented by minced meat, beef burger, kofta and sausage (50 of each) collected from different shops and supermarkets at El Menofiya and Cairo Governorates at different periods of time. All collected samples were examined bacteriologically as rapidly as possible for determination of their contamination with Pseudomonas species bacteria as well as detection of their virulence factors using multiplex PCR technique

Bacteriological examination:

Samples Preparation ***(FDA, 2002):*** Under complete aseptic conditions, 25 grams of the sample were weighed and transferred into a sterile homogenizer flask containing 225 ml of sterile peptone water (0.1%). The content of the flask was homogenized for 3 minutes at 14000 rpm then allowed to stand for 5 minutes at room temperature.

2.2.2 Determination of Pseudomonas spp. ***(ISO, 2004)*** accurately, 0.1 ml of each sample homogenate was separately inoculated into duplicate Petri-dishes of Pseudomonas selective agar medium base supplemented with glycerol and evenly spread. The inoculated plates were incubated at 25 °C for 48 hours after which all developed colonies (greenish yellow colonies) were enumerated. The average count was calculated and recorded.

2.2.3 Identification of isolated Pseudomonas species: The suspected colonies were purified and subcultured on nutrient agar slopes and incubated at 37°C for 24 hours. The purified colonies were subjected for further identification including morphological and biological identification according to Macfaddin (2000).

Polymerase Chain Reaction (PCR)

Genomic DNA extraction: Using GeneJET Genomic DNA Purification Kit DNA amplified products "PCR master Mix" (Fermentis): Gel Elecrophoresis: Sambrook *et al.,* (1989).

Primer sequences of *P. aeruginosa* used for PCR system:

Accurately, the application of PCR for identification of gryase B (*gyrB*), exotoxin A (*ETA*) and outer membrane protein (*oprL*) *genes of P. aeruginosa* was adopted by using certain primers as shown in the following table:

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
gyrB (F)	5′ CCTGACCATCCGTCGCCACAAC ′3	222	**Motoshima *et al.*, (2007)**
gyrB (R)	5′ CGCAGCAGGATGCCGACGCC ′3		
ETA (F)	5′ GACAACGCCCTCAGCATCACCA ′3	397	**Khan and Cerniglia (1994)**
ETA (R)	5′ CGCTGGCCCATTCGCTCCAGCG ′3		
oprL (F)	5′ ATGGAAATGCTGAAATTCGGC ′3	504	**de Vos et al. (1997)**
oprL (R)	5′ CTTCTTCAGCTCGACGCGACG ′3		

Amplification reaction of P. aeruginosa
(Salman *et al.*, 2013)

Each 50µl of the multiplex PCR mixture, in addition to the template DNA, contained 10 x buffer 5 µl, 1.5 mM MgCl2, 0.4mM of each dNTP, 5U of Taq DNA polymerase (Fermentas, USA), 0.25 µM of the primers targeting oprL gene and 0.5µM of each of the primers targeting, gyrB, ETA and 16S rDNA gene fragments.

The thermal cycler conditions for the multiplex PCR were: initial denaturation step at 94°C for 5 minutes followed by 35 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1.5 minutes; and a final extension step at 72°C for 7 minutes. Similar multiplex PCR conditions were applied to the DNA templates of negative control isolates. The amplified products were electrophoresed on 2% agarose gel, stained with ethidium bromide (5 µg /100 ml) and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder was used to determine the fragment sizes

.

RESULTS

Table (1): Incidence of Pseudomonas species in the examined samples of meat products

Meat products	No. of examined samples	No.	%
Minced meat	50	9	18
Beef burger	50	15	30
Beef Kofta	50	13	26
Beef Sausage	50	11	22
Total	200	48	24

Table (2): Incidence of identified Pseudomonas species in the examined samples of meat products (n=50 of each).

Meat products / *Pseudo-monas* strains	Minced meat		Beef burger		Beef Kofta		Beef Sausage	
	No.	%	No.	%	No.	%	No.	%
P. aeruginosa	2	4	4	8	2	4	3	6
P. diminuta	2	4	3	6	8	16	2	4
P. fluorescens	4	8	2	4	3	6	7	14
P. proteolytica	1	2	1	2	2	4	1	2
P. fragi	-	-	5	10	5	10	8	16
P. putrefaciens	1	2	1	2	3	6	-	-
P. putida	-	-	5	10	2	4	2	4
Total	10	20	21	42	24	48	23	46

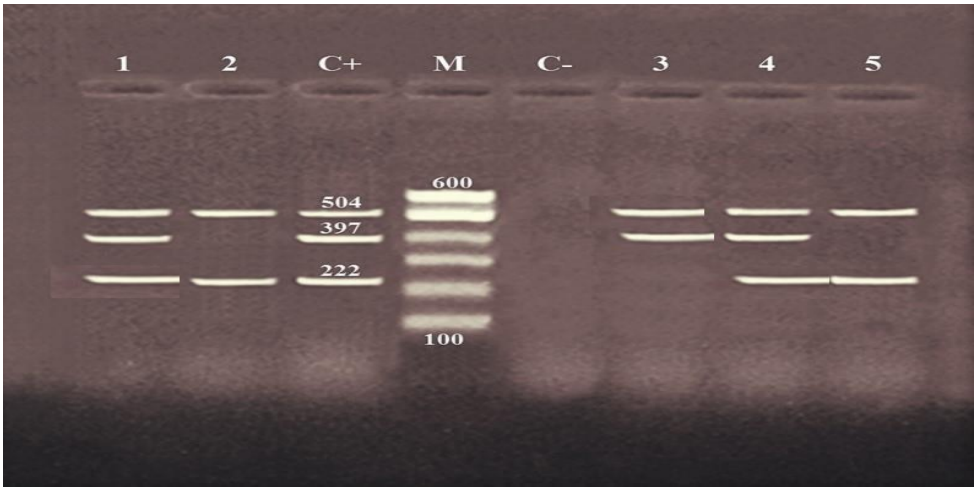

Fig. (1): Agarose gel electrophoresis of multiplex PCR of gyrB (222 bp), ETA (397 bp) and oprL (504 bp) genes for characterization of Pseudomonas aeruginosa. **Lane M:** 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *P. aeruginosa* for *gyrB*, *ETA* and *oprL* genes.
Lane C-: Control negative.
Lane 1& 4: Positive *P. aeruginosa* strain for *gyrB*, *ETA* and *oprL* gene
Lane 3: Positive *P. aeruginosa* strain for *oprL* genes *and ETA* gene.
Lanes 2 & 5: Positive *P. aeruginosa* strains for *gyrB* and *oprL* genes.

Table (3): Prevalence of virulence genes of *P. aeruginosa* strains isolated From the examined samples of meat products (n= 11 strains).

Target gene	No.	%
ETA	4	36
gyrB & *oprL*	6	54
ETA & *oprL*	2	18
gyrB, *ETA* & *oprL*	2	18
Total	11	100

***ETA*:** exotoxin A gene.
***gyrB*:** gryase B gene.
***oprL*:** outer membrane protein gene.

DISCUSSION

Pseudomonas species are Gram-negative pathogens which responsible for 2 million annual hospital-acquired infections, adding tremendously to U.S. healthcare cost. *Pseudomonas aeruginosa*, an opportunistic human pathogen, is commonly associated with nosocomial infections, particularly ventilator-associated infections and pseudomonal pneumonia in immune compromised patients with cystic fibrosis, chronic obstructive pulmonary disease, ventilator-associated pneumonia, community-acquired pneumonia, and bronchiectasis (Bomberger *et al.*, 2009).

Results obtained in table (1) revealed that the incidence of Pseudomonas species in the examined samples (minced meat, beef burger, kofta and sausage) were (18% (9) ,30% (15), 26% (13) and 22% (11)) respectively. incidence of isolation of Pseudomonas in the examined minced meat was (18%(9) higher incidence recorded by Amal *et al.,* (2014) who isolated pseudomonas by the percentage of 32 (71.11%) and on the other hand lower incidence reported by Tassew *et al.,* (2010) isolated pseudomonas by the percentage of 9 (5.5%).While in the examined beef burger was 30%(15) comparatively lower results obtained by Amal *et al.,* (2014) isolated pseudomonas by the percentage of (26.67%) and Sofy *et al.,* (2017) isolated pseudomonas by the percentage of (1%).on the other hand in the examined kofta was 26%(13) comparatively lower results obtained by Tassew et al. (2010) isolated *pseudomonas spp*.by the percentage of 9 (5.5%).and the same result reported by Hosseini *et al.,* (2008) who isolated *pseudomonas spp* from kofta by the percentage of (55%).finally in the examined sausage was 22% (11) comparatively lower results obtained by Sofy *et al.,* (2017) isolated pseudomonas by the percentage of (5%).

Meat products are liable to harbor different types of microorganisms through a long chain of handling, processing, distribution and storage as well as preparation. Within this

respect, they are considered as serious sources of food borne diseases and have been frequently linked to major outbreaks of food poisoning all over the world (Hassanien, 2004) Meat spoilage remains as an unsolved problem for the meat industry. This will result in imperative financial misfortunes, nourishme nt waste and misfortune of buyer certainty within the meat showcase. Gram-negative microbes included in meat deterioration are aerobes or facultative anaerobes. These speak to the bunch with the most prominent meat deterioration potential, where Pseudomonas tend to rule the microbial consortium beneath refrigeration and high-impact conditions. (Mohareb *et al.*, 2015).

Results given in table (2) revealed that the incidence of identified pseudomonase species in the examined minced meat were *P. aeruginosa* 4 % (2), *P. diminuta* 4% (2), P.*fluorescens* 8% (4) ,*P. proteolytica* 2%(1) and *P. putrefaciens*2%(1) . these results dis agree with Ercolini *et al.,* (2010) isolated *P. Fragi* and agree with Ukut *et al.,* (2010) isolated *Pseudomonas aeruginosa* (4(11.1%)) and Pseudomonas spp. (3(8.3%).while, in beef burger were *P.aeruginosa , P. diminuta ,P.fluorescens P. proteolytica,P.fragi P. putrefaciens and P. putida* with respective incidence of (8%(4) ,6%(3) , 4%(2) , 2%(1), 10%(5) , 2%(1) and 10%(5). these results agree with De Jonghe *et al.,* (2011) isolated *P. fragi, P. fluorescens and P. putida* and agree Amal *et al.,* (2014) isolated *P.aeruginosa.*and dis agree with Andreani and Fasolato, (2017) who isolated *Pseudomonas, Shewanella* and *Xanthomonas. On* the other hand in kofta were *P.aeruginosa , P. diminuta ,P.fluorescens P. proteolytica,P.fragi P. putrefaciens and P. putida* with the incidence of (4%(2) ,16%(8) , 6%(3) , 4%(2), 10%(5) , 6%(3) and 4%(2), respectively. these results agree with Mohareb et al. (2015) isolated *Pseudomonas putida* and agree with De Jonghe et al. (2011) who isolated *Pseudomonas fragi, P. fluorescens* and P. *putida. also* disagree with Easa, (2010) who isolated *Pseudomonas putrefaciens.* Finally in sausage *P.aeruginosa , P. diminuta ,P.fluorescens P. proteolytica,P.fragi and P. putida* with respective incidence of (6%(3) ,4%(2) , 14%(7) , 2%(1), 16%(8) and 4%(2). These results agree with Bukhari and Aleanizy (2019) who isolated *P. aeruginosa* and agree with Ercolini *et al.,* (2010) who isolated *P. fragi.* Also agree with Akan and Gürbüz (2016) who isolated *P. aeruginosa*, *P. fluorescens* and *P. putida.*

These results indicate that most of the meat products samples examined did not meet the quality standards, will render these foods unfit for human consumption. Also, the examined samples have pathogenic bacteria which make these not satisfactory during public health standard. Enough these organisms will cause infection and intoxication that rise potential risks to consumers. Finally, the relevant authorities should draw the attention towards the health education campaign on food safety.in addition to, food handlers should receive training on safety principles of good hygiene practice. Good regulations in safe production, proper processing, and consumer awareness is highly recommended. People must apply theses participation. (Sofy *et al.,* 2017). The presence of these species in meat products make it may be stated that hygienic conditions should be checked in production, packaging, transport, storage and marketing of meat and meat products. Akan and Gürbüz, (2016). the presence of microorganisms like *P. putida, P. aeruginosa, Pseudomonas fragi* and *P.fluorescens* which can lead to a quick spoilage of the products and reduced shelf life of them .. Efforts made by the Government to improve the microbiological quality of fresh beef should deal not only with the respect of good slaughtering, evisceration, and hygienic practices but also with the cleaning process of surfaces where meat is cut and sold (Mouafo *et al.,* (2020).

Meat spoilage is usually associated with *P. fluorescens, P. aeruginosa, P. fragi, and P. lundensis.* (Caldera *et al.,* 2016) in addition to this, the ability of these spoilage bacteria to survive under low temperatures may cause difficulty during the storage of foods (Bellés et al. 2017; Wang *et al.,* 2017).

Presence of *Pseudomonas spp*. in food samples is of great significance as the organism is considered as a pathogenic bacterium for man and as an indicator of food quality, Yagoub, (2009). Apart from being a spoilage microorganism, *Pseudomonas spp.* could cause urinary and blood stream infection Golemi-Kotra, (2008).

Results obtained in table (3) revealed that the incidence of *P. aeruginosa* strains isolated from the examined samples of meat products (n= 11

strains).Were exotoxin A gene (ETA)36%(4) ,gryase B gene (gyrB)with outer membrane protein gene (oprL)54%(6) ,exotoxin A gene (ETA) with outer membrane protein gene (oprL)18%(2) and gryase B gene(gryB) with exotoxin A gene (ETA) and outer membrane protein gene (oprL) 18%(2).

These results disagree with Ercolini *et al.*, (2007) detected the carbamoyl phosphate synthase gene (*carA*) in different species of pseudomonas.

CONCULSION

The presence of *Pseudomonas spp*. should to be avoided amid prior stages
of nourishment planning. Additionally, during serving, temperature abuse will lead to spoilage of food leading to bad odor and taste, which is not palatable for customers that can affect sales and reputation of the food service establishments. Isolates of *P. aeruginosa* have virulence-associated genes. It is important to give more attention to *P. aeruginosa* because they are able to produce toxins, grow under low temperatures and broad spectrum of environments so hygienic measures should be adopted to control microbial contamination.

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