Benha Veterinary Medical Journal 39 (2020) 47-51

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Phenotypic and genotypic characterization of pseudomonas species isolated from frozen meat

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ARTICLE INFO

Keywords

AB susceptibility
Frozen meat
Minced meat
PCR.
Pseudomonas
Psychrotrophic bacteria

Received 18/10/2020
Accepted 29/10/2020
Available On-Line 20/01/2021

ABSTRACT

One hundred samples of imported Brazilian frozen meat (50 frozen cubic meat and 50 minced meat) were collected from different supermarkets in El-Menoufia Governorate, Egypt to be examined bacteriologically for detection of Pseudomonas species. The incidence of Pseudomonas species were (35/50) 70% in frozen cubic meat. While, the incidence of such organism in the examined frozen minced meat samples was 80% (40/50). Psychrotrophic bacterial count in the examined frozen cubic meat ranged from 6 x 10^2 to 1.9 x 10^5 with mean value 2.24 x 10^4 cfu/g. In addition, Psychrotrophic bacterial count in frozen minced meat ranged from 7 x 10^2 to 9 x 10^5 with mean value 1.7 x 10^5 cfu/g. The incidence of identified Pseudomonas species (number and percentages) detected in the examined samples of frozen meat represented by *Ps. aeruginosa*, *Ps. fluorescence*, *Ps. diminuta*, *Ps putida* and *Ps. fragi* were 15(30%), 40(80%), 8(16%), 5(10%) and 4(8%), respectively. Regarding the minced meat samples the incidence of identified *Ps. aeruginosa*, *Ps. fluorescence*, *Ps. diminuta*, *Ps. putida*, *Ps. fragi* were 20(40%), 45(90%), 5(10%), 7(14%) and 8(16%), respectively. The *Pseudomonas* species were resistant to Oxacillin. They were sensitive to Gentamycin except *Ps. fluorescence.* PCR is rapid and reliable tool for identification of different bacterial species.

1. INTRODUCTION

Meat and meat products are very important foods for human being due to their palatability and high nutritional value. They are important sources for protein, with good balance of essential amino acids, minerals, B-complex vitamins and other nutrients (Hui *et al.* 2001). The increased in demand for frozen meat arises resulting from Egypt's new policy and development of international trade of foods to provide frozen meat at reduced affordable prices (GOVS, 2004). Thus, imported beef may be the main chance to bridge the gap between the consumption and domestic supply. Pseudomonas species are considered the most important Psychrotrophic microorganisms causing meat spoilage (Lu and Bi, 2007). Psychrotrophic bacteria grow well at or below 7°C and have their optimum temperature for growth between 20-30°C. Pseudomonas species are opportunistic Gram-negative pathogens that cause outbreak upon the change in normal environmental conditions to unhygienic one (Roberts, 2001).

Massive use of antibiotics in food producing animals can develop antibiotic resistance in the intestinal bacteria. Further, the antibiotic resistance genes may transfer bacteria to disease causing bacteria resulting in antibiotic resistant infections for humans (Serrano, 2005).

Therefore, this study aimed to determine the incidence of pseudomonas species in frozen meat (cubic and minced) through estimation of Psychrotrophic counts, isolation and identification of Pseudomonas species in frozen meat. In addition, determination of their antibiotic susceptibility was kept in consideration.

2. MATERIAL AND METHODS

2.1. Collection of meat samples:

A total of 100 random samples of imported frozen meat (n=100) (50 cubic meat and 50 minced meat) were randomly collected from different retail shops and supermarkets in El-Menoufia Governorate, Egypt to be bacteriologically examined for detection of phenotypic and genotypic of Pseudomonas species.

2.2. Preparation of samples:

The examined samples were thawed at refrigerator under complete aseptic conditions. After that, 25 g of each examined sample were weighed aseptically by sterile scissor and forceps from the central part after surface sterilization by hot spatula then placed into separate sterile polyethylene bag to which, 225 ml of 0.1% sterile buffered peptone water were aseptically added to the content of bag .Thereafter, each sample was homogenized in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One ml from the original dilution was transferred by sterile pipette to another sterile tube having 9ml sterile buffered peptone water 0.1% then mixed well to make the next dilution, to make tenfold decimal serial dilutions.

2.3. Determination of Psychrotrophic count (FAO, 1992):

Accurately 0.1 ml from each of the previously prepared serial dilution was spread over dry surface of plate count agar medium plates (CM0463-OXOID) by sterile bent glass spreader. Then inoculated and control plates were incubated

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at 7 °C for 10 days .Then all colonies were counted, and the average number of colonies was recorded. And the total psychrotrophic bacterial count (cfu/g) was calculated.

2.4. Isolation and Identification of Pseudomonas:
The suspected colonies were purified and sub-cultured onto nutrient agar slopes (EB0336-OXOID) and incubated at 37°C for 24hrs. The purified colonies were subjected for further identification microbiologically as following Gram stain (*Cruickshank etal*.,1975) , Motility test (MacFaddin 2002) and biochemically according to (Collins and Lynne 1984 and MacFaddin 2002) as following:

1. Pigment formation on nutrient agar (Collins and Lyne, 1984).
2. Catalase test (Kiss, 1984)
3. Sugar fermentation test (MacFaddin, 1976)
4. Nitrate reduction test (Kiss, 1984)
5. Indol production test (Bialy and Scott, 1978)
6. Methyl red test (Cruick Shank et al., 1975)
7. Voges – proskaure test (Cruick Shank et al., 1975)
8. Cytochrome oxidase test.
9. Growth at 42 °c and 4 °c (Collins and Lyne, 1984)
10. Arginine hydrolysis (Collins and Lyne, 1984)
11. 10% lactose agar test (Washington, 1981)
12. Starch hydrolysis test (Kiss, 1984)
13. Oxidation and fermentation test (Cruickshank et al., 1975)
14. Hydrogen sulfide (H2S) production test (Washington, 1981)
15. Gelatin liquefaction test (Cowan and steel, 1979).

2.5. Antibiotic susceptibility test of Pseudomonas isolates:
Five isolates of each pseudomonas species were submitted for antibiotic sensitivity test. Antibiotic susceptibility was performed by Disc diffusion method according to (Schreckenberger and Binnicker, 2011).

Samples were processed for culture and sensitivity pattern for Pseudomonas colonies were picked up (using a sterile loop) to tube containing 5 ml of Muller-Hinton broth. The broth culture was incubated at 37°C for 24 hours. Then the turbidity was adjusted to the turbidity of the 0.5 McFarland standards. After that about 1ml of the broth was inoculated on the surface of Muller-Hinton agar (CM0337-OXOID) and spread evenly over the entire surface of the agar plates by a sterile bended glass rod. The antibiotic discs of Amikacin, Cefepime, Ceftazidime, Ceftriaxone, Gentamycin, Ciprofloxacin, Imipenem, Oxacillin and Tobramycin were applied on the inoculated plates. Then, the plates were placed in an incubator at 37°C for 18 h in inverted position. After 18 hours of incubation, the plates were examined and the diameters of zone of inhibition were measured in mm

Table 1 The used antimicrobial agents in the antibiotic sensitivity test

Antimicrobial agents	Concentration	company	Catalog number
Amikacin (AK)	30µg	Oxoid™	CT0107B
Cefepime (FEP)	30µg	Oxoid™	CT0771B
Ceftazidime (CAZ)	30µg	Oxoid™	CT0412B
Ceftriaxone (CRO)	30µg	Oxoid™	CT0417B
Ciprofloxacin (CIP)	5µg	Oxoid™	CT0425B
Gentamycin (CN)	10µg	Oxoid™	CT0024B
Imipenem (IPM)	10µg	Oxoid™	CT0455B
Oxacillin (OX)	1µg	Oxoid™	CT0159B
Tobramycin(TOB)	10µg	Oxoid™	CT0056B

2.6. Antibiotic resistance genes
PCR was carried on a T100 thermal cycler (Bio-rad) with temperature and time conditions shown in Table (2) according to (References of primers) and Dream Taq green Master Mix (Thermo scientific™, K1081).

Table 2 Sequences, target and expected amplicon size for forward and reverse primer pairs used in the study.

Primer	Sequence	Amplified product	Reference
RpoB	F:CAGTTCATGGACCAGAACAACCCG	759	*Benie CK etal. (2017)*
	R: ACGCTGGTTGATGCAGGTGTTC		
OprL	F: ATG GAA ATG CTG AAA TTC GGC	396	*Mohammed Gihan etal. (2015)*
	R: CTT CTT CAG CTC GAC GCG ACG		
ToxaA	F:GACAACGCCCTCAGCATCACCAGC	504	
	R: CGCTGGCCCATTCGCTCCAGCGCT		

3. RESULTS

The total Psychrotrophic bacterial count in the examined frozen cubic meat ranged from 6×10^{2} to 1.9×10^{5} with mean value 2.24×10^{4} cfu/g. In concern to the examined frozen minced meat, it ranged from 7×10^{2} to 9×10^{5} with mean value 1.7×10^{5} cfu/g (Table 3).
The incidence of pseudomonas species were (35/50) 70% in examined frozen meat cubics (n=50) as 35 samples were positive and each one applied on 3 slopes totally 105 isolates (35 × 3). While, in the examined frozen minced meat samples (n=50) were (40/50) 80% as 40 samples were positive, and each applied on 3 slopes totally 120 isolates (40 × 3) (Table 4).
The incidence of pseudomonas species isolated from frozen examined meat samples were *Ps. aeruginosa, Ps. fluorescence, Ps. diminuta, and Ps. putida and Ps. fragi* (Table 5).

Results of antibiotic sensitivity tests against Ps. aeruginosa is demonstrated in table (6).

Table 3 Total Psychrotrophic bacterial count in the examined frozen meat (cfu/g) (n=50 of each).

Type of meat	Minimum	Maximum	Mean value ± S.E*
Frozen cubic meat	6×10^{2}	1.9×10^{5}	$2.24\times 10^{4}\pm 3.56\times 10^{4}$
Frozen minced meat	7×10^{2}	9.0×10^{5}	$1.70\times 10^{5}\pm 3.07\times 10^{5}$

S.E* = standard error of mean.

Table 4 Incidence of pseudomonas species isolated from examined frozen cubic and minced meat samples (50 of each).

Meat sample	No. of examined samples	No. of +ve samples	%*
Frozen cubic meat	50	35	70%
Frozen minced meat	50	40	80%
Total	100	75	75%**

*Percentage in relation to No. of samples for each type of meat (n=50). ** Percentage in relation to No. of samples (n=100).

Table 5 Incidence of pseudomonas species isolated from examined samples meat and minced meat samples (n=50 of each).

Type of meat	Frozen meat cubic		Frozen minced meat	
	No.	%*	No.	%*
Ps. aeruginosa	15	30	20	40
Ps. fluorescence	40	80	45	90
Ps. diminuta	8	16	5	10
Ps. putida	5	10	7	14
Ps. fragi	4	8	8	16

* Percentage in relation to total No. of each type and samples (n=50).

Ps. aeruginosa were sensitive to Gentamycin, Tobramycin, Cefepime, Ceftazidime, Imipenem, Amikacin and ciprofloxacin. Ciprofloxacin is the most potent drug available for the treatment of *ps. aeruginosa* infections .*Ps. aeruginosa* were resistant to Ceftriaxone and Oxacillin. *Ps. aeruginosa* is considered to be one of the most dangerous species as it forms biofilms, which aids in colonization of food spoilage and increases resistant to antibiotics, disinfectants and antiseptics.

Also, the obtained results indicated that *Ps. fluorescence* are more resistant to Amikacin, Oxacillin, Cefepime and Ceftazidime Gentamycin and Ciprofloxacin. Actually *Ps. diminuta* was more sensitive to Amikacin, Ceftazidime and Tobramycin, however, it was resistant to Cefepime, Ciprofloxacin, Gentamycin, Imipenem and Oxacillin.

Pseudomonas putida was resistant to Amikacin, Oxacillin, and also was sensitive to Cefepime, Ceftazidime, Ciprofloxacin, Gentamycin, Imipenem, Ceftriaxone and Tobramycin.

Furthermore, the results showed that *Ps. fragi* was more resistant to Oxacillin, Amikacin, Ceftazidime and Tobramycin. While it was sensitive to Cefepime, Ceftriaxone, Imipenem, Ciprofloxacin and Gentamycin.

Table 6 Results of antibiotic sensitivity tests against *Ps aeruginosa*.

Antimicrobial Agent	*Ps. Fluorescence*	*Ps. aeruginosa*	*Ps. Putida*	*Ps. diminuta*	*Ps. fragi*
Amikacin (AK)	R	S	R	S	R
Cefepime (FEP)	R	S	S	R	S
Ceftazidime (CAZ)	R	S	S	S	R
Ceftriaxone (CRO)	R	R	S	I	S
Ciprofloxacin (CIP)	R	S	S	R	S
Gentamycin (CN)	R	S	S	R	S
Imipenem (IPM)	R	S	S	R	S
Oxacillin (OX)	R	R	R	R	R
Tobramycin(TOB)	R	S	S	S	R

R: resistant, S: sensitive, I : intermediate

The recorded virulence genes for some *Ps. aeruginosa* strains isolated from the examined frozen meat samples by PCR (n: 1-4 frozen meat and 5-8 minced meat) is shown in table (7) and fig. (1)

Table 7 Virulence genes for *Ps. aeruginosa* strains isolated from the examined frozen meat samples by PCR (n: 1-4 frozen meat and 5-8 minced meat)

Isolation No	rpoB	Oprl	ToxaA
1	+	+	
2	-	-	-
3	+		
4	-	-	-
5	+		
6	+		
7	-	-	-
8	-	-	-

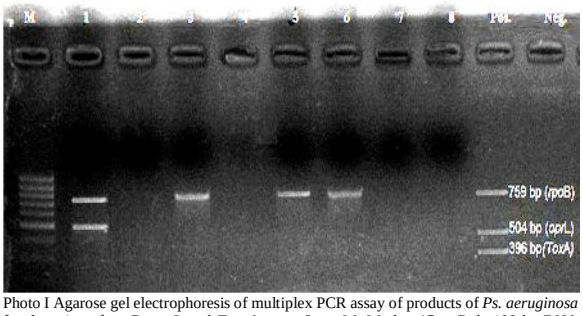

Photo I Agarose gel electrophoresis of multiplex PCR assay of products of *Ps. aeruginosa* for detection of *rpo*B, *opr*L and *Toxa*A genes.Lane M: Marker (GeneRuler100 bp DNA ladder, Thermo scientific™). Lanes 1,3,5,6 Positive for *rpo*B gene with 759 bp amplicon. Lane 1 Positive for *opr*L gene with 504 bp amplicon. Lanes 2, 4, 7, 8: negative for *rpo*B, *opr*L and *Toxa*A genes. Pos.: Positive control (*reference strain Ps. aeruginosa* obtained from Central Laboratory, Faculty of Veterinary Medicine, Benha University), Neg.: Negative control

4. DISUCSSION

The imported frozen meat is often contaminated by spoilage microorganisms that responsible for changes in meat (Tauxe, 2002).

Freezing of meat depend on retardation of microbial growth to the point where decomposition due to microbial action does not occur (ICMSF, 1978).

The hygienic measures are still not well controlled, and the growth of spoilage may manifest itself as visible growth, textural changes and off -odors or off-flavors. Such changes make foods unpalatable and unfit for human consumption (Dave and Ghaly, 2011).

Pseudomonas species are psychrotrophic organisms that transfer to frozen meat during handling, transportation, storage and from unsanitized equipment (Venugopal *et al*., 1984).

The results showed that the psychrotrophic bacterial count (cfu/g) in the examined samples of frozen minced meat ranged from (7×10^2) to (9×10^5) with mean value (1.7×10^5). Accordingly, all examined samples were accepted according to EOS (2005) which reported that the maximum limit for psychrotrophic bacterial count in imported frozen meat shouldn't exceed 10^6 cfu/g. Higher results were obtained by Karaboz and Dincer (2002), Badawi (2008), Ibrahim *et al*, (2011) and Ali (2016) in the examined frozen meat those found that average count ($2.18 \times 10^4 \pm 0.1 \times 10^4$)cfu/g ,(43×10^3) cfu/g with mean value ($9 \times 10^5 \pm 6.1 \times 10^5$) sample from Alex Seaport , (9.9×10^2 to 4.2×10^6) with mean value ($3.7 \times 10^5 \pm 7.9 \times 10^4$) cfu/g and (9.9×10^2 to 4.2×10^6) cfu/g with mean value ($3.7 \times 10^5 \pm 7.9 \times 10^4$) cfu/g , respectively. Also Abd El-Hady (2014) reported that psychrotrophic count in imported frozen beef chunk and ribs were 1 x 10^3 to 1.8x 10^5 with mean value $2.78 \times 10^4 \pm 5.24 \times 10^3$ cfu/g , 1.7×10^3 to 7.9×10^5 with mean value $3.87 \times 10^4 \pm 4.35 \times 10^3$ cfu/g, respectively. With incidence of 24 (96%), 24 (96%) respectively.

The results showed that the incidence of Psychrotrophic bacteria were higher in frozen minced meat than frozen meat. The incidence of *Pseudomonas* species isolated from the examined samples was 70%, 80% in frozen cubic meat and minced meat, respectively. The incidence of *Ps. areuginosa, Ps. fluorescence, Ps.diminuta, Ps. putid and Ps.fragi* in frozen meat were 15(30%), 40(80%), 8(16%), 5(10%) and 4(8%), respectively. In addition, the incidence of *Ps. aeruginosa, Ps. fluorescence, Ps. diminuta, Ps. putida and Ps. fragi* were 20(40%), 45(90%), 5(10%) 7(14%) and 8(16%), respectively.

Lower results were obtained by El Nawawi *et al.*,(2012) , Hassan (2013), Abd Elaal (2017) and also El-Dakrory

(2017) who found that *ps. aeruginosa* in imported frozen meat was (6%), (4%) ,(12.2%),(7.1%), respectively. While Rizk (2014) reported that the incidence of *Ps. fluorescence* was (30%), while *Ps. aeruginosa* was not isolated. Higher results for isolation of *Ps. fluorescence* were obtained by Abd Elaal –Mohga (2017), El-Dakrory (2017) and Ibrahim, *et al*., (2016) those explained that the incidence of *Ps. fluorescence* in imported frozen meat was 64.44%, 46.67% and 73.33%, respectively. Abd Elaal (2017) and *Ibrahim et al.* (2016) those reported that the incidence of *Ps. putida* in imported frozen meat was (8.34%) and (8.34%) respectively.

The results of Abd Elaal (2017) and El-Dakrory (2017) showed that the incidence of *Ps. fragi* in imported frozen meat was (8.34%) and (28.27%), respectively.

The presence of pseudomonas species in food represents risk as they lead to spoilage of food (Jay, 2000). *Ps. fluorescence* and *Ps. aeruginosa* represented the major species which could be isolated due to its resistance to many stress factors as low temperature, water activity and inhibitory action of carbon dioxide. These finding agreed with El-Dakrory - Amira (2017).

Antibiotics are massively used in beef industry which increases the risk of developing antibiotic resistant microorganisms, Therefore, the isolated Pseudomonas species were submitted to antibiotic susceptibility testing.

The results showed that *Ps. aeruginosa* were sensitive to Gentamycin, Tobramycin, Cefepime, Ceftazidime, Imipenem, Amikacin, Tobramycin and ciprofloxacin which nearly results were approved by *Gales et al. (2001),* who reported that Ciprofloxacin is one of the most potent drug available for the treatment of *ps. aeruginosa* infections. *Ps. aeruginosa* was resistant to Ceftriaxone and Oxacillin.

Also, the results showed that *Ps. fluorescence* are more resistant to Amikacin, Oxacillin, Cefepime and Ceftazidime Gentamycin and Ciprofloxacin which nearly results recorded by Morgan (2014).

Ps. putida was resistant to Amikacin, Oxacillin and Ceftriaxone. And also, it was sensitive to Cefepime, Ceftazidime, Ciprofloxacin, Gentamycin, Imipenem and Tobramycin. These results were nearly similar to those reported by Muller (2011).

In addition, the results showed that *Ps. fragi* was more resistant to Oxacillin, Amikacin, Ceftazidime and Tobramycin.

While it was sensitive to Cefepime, Ceftriaxone, Imipenem, Ciprofloxacin and Gentamycin.

Pseudomonas species have multiple intrinsic and acquired resistance genes, host several mobile genetic elements and exchange them with other families of Gram-negative bacilli. *(Pfeifer et al., 2010).*

The reason for increased antimicrobial resistant was the overuse of antimicrobial drugs for preventing or treating infections in human and veterinarian (WHO, 2007).

PCR is rapid and reliable tool for identification of different bacterial species (Settanni and Corsetti, 2007).

The PCR results for virulence genes of *ps. aeruginosa* for detection of (oprl, rpoB, ToxaA) detected that oprl virulence gene were showed in 1 from 8 strains (12.5%) with 504 bp amplicon, toxaA virulence gene were absence in 8 strains, rpoB virulence gene were showed in 4 from 8 strains (50%) with 759 bp. 2 samples in frozen meat and 2 samples from minced meat.

5. CONCULOSIONS

The examined imported frozen meat samples were highly contaminated with various species of pseudomonas reflecting unhygienic measures and unsuitable environmental condition during handling, transportation and storage. Pseudomonas species are the most psychrotrophic bacteria contaminated the examined samples. The examined samples were highly contaminated with *Ps. fluorescence* followed by *Ps. aeruginosa*. The rpoB virulence gene were present in 4 strains of *Ps. aeruginosa* (50%) then oprl virulence gene (12.5%) and absence of Toxa A virulence gene in 8 strains.

5. REFERENCES

1. Abd Elaal, M. A. (2017) Prevalence of pseudomonas species in imported frozen meat with special reference *to ps. aeruginosa*. M.V.SC., Thesis (Meat Hygiene), Fac. Vet. Med., Benha University.
2. Abd El-Hady,N. T. (2014): Psychotrophic spoilage organisms in imported frozen beef cuts Ph.D. Thesis (Meat Hygiene), Fac. Vet. Med., Alexandria University.
3. Ali, M. (2016) Bacterial Contamination of Imported Frozen Meat M.V. Sc., Thesis (Meat Hygiene), Fac. Vet. Med. Alexandria University.
4. Badawi, M. (2008): Further studies on imported frozen meat Ph.D. Thesis (Meat Hygiene), Fac. Vet. Med., Alex. University.
5. Bailley, W.R and Scott, E.G. (1978): Diagnostic Microbiol .A textbook for the isolation and identification of pathogenic Microorganisms. The C.V. Mosby Company, Saint Louis.
6. Benie, C.K; Dadié A,; Guessennd N, N. ;gbesso-Kouadio, N.A.; Kouame, ND; N'golo, D.C.; Aka S. ; Dako, E.; Dje, K.M. and Dosso, M. (2017). Characterization of Virulence Potential of *Ps. aeruginosa* Isolated from Bovine Meat, Fresh Fish, and Smoked. European Journal Of Microbiology And Immunology 7(1):55-64
7. Collins, C.H. and Lyne P.M. (1984): Microbiological Methods.5th Microbiology Laboratory Manuals .British Library Butterworth Inc .Co.
8. Cowan, S.T. and Steel, K.J. (1979): Manual for the Identification of Medical Bacteria .2nd Ed .Cambridge Univ.Press, England.
9. Cruickshank, R.; Duguid, J.P.; Marmion, B.P.and Swain, R.H.A. (1975): Medical Microbiology, 12th ed. Churchill living stone Edinburgh, London and New York.
10. Dave, A.R. and Ghaly, A.E. (2011): Meat spoilage mechanisms and preservation technique .Department of Process Engineering and Applied Science, Dalhousie University, Halifax Nova Scotia, Candada.
11. E.S "(Egyptian Organization for Standardization and Quality Control: 2005): Egyptian Standard Specification of frozen meat No.1522.
12. El –Nawawi, F.A.; Attala, O.A. and Saleh, S. (2012): Enteric pathogen of public health importance in imported frozen meat and chicken, Int.J. Of Microbiology Research. 3(1):59-63.
13. El-Dakrory, A., (2017): Psychrotrophic microorganisms in frozen meat .M.V.Sc., Thesis (Meat Hygiene), Fac. Vet. Med., Benha University.
14. FAO "Food and Agriculture Organization "(1992): Manual of Food Quality Control . 4Rev.1.Microbiological analysis (Andrews, W.Ed) FAO food and nutrition paper No.14/4 Rev.
15. Gales, A.C.; Jones, R.N.; Turnidge, J.; Renee, R. and Ramphal, R. (2001): Characterization of *ps. aeruginosa* .occurrence rate, antimicrobial susceptibility pattern and molecular typing in the global sentry antimicrobial surveillance program. Clinic .Infect. Dis. 32:S146-55.
16. GOVS '; General Organization for Veterinary Service" (2004): General management for slaughtering and general management for quarantine and inspection.

17. Hassan, M.A. (2013): Bacterial associated with fungal contamination in frozen beef meat .M. V. Sc. Thesis Microbiology .Fac. Vet. Med. Alex .Univ.
18. Hui, Y.H.; Nip, W.R; Rogers R.W. and Youn, O.A. (2001): Meat science and application .Marcel Bekker, NewYourk. https://doi.org/10.1201/9780203908082
19. Ibrahim, M, A. R. (2011): Microbiological evaluation of retail frozen meat .M.V.SC., Thesis , Meat Hygiene, Fac. Vet. Med. Alex University.
20. Ibrahim, He.M.; Hassan, M.A.; Abou El-Roos, N. and Abd Elsalam, M.A. (2016): Prevalence and molecular characterization Pseudomonas species in frozen meat .B.V.M.J., 31(2):220-224.
21. ICMSF "International Commission on Microbiological specification for food" (1978): Microorganisms in food, their significance and method of enumeration .2nd Ed. Univ. Of Toronto Press, Toronton and Buffallos Canada
22. Jay, J.M. (2000): Food preservation with modified atmospheres 283:205.I.N.D.R.Hoidnian led Modern food microbiology Aspen Publieshers, Inc.Gaaithcrsbura .Med.
23. Karaboz, I.and Dincer, B. (2002): Microbiological investigation on some of the commercial frozen meat in IZMIR. Turkish Electronin. J.Biotecnol. Special issue, 18-23.
24. Kiss, J. Z., Mezey, E., and Skirboll, L. (1984). Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. Proceedings of the National Academy of Sciences, 81(6):1854-8. doi: 10.1073/pnas.81.6.1854.
25. Lu, Y. and Bi, Z. (2007): Potential use of a transposon Tn916-generated mutant of *Aeromonas hydrophila* J-1 defective in some exoproducts as a live attenuated vaccine. Prev. Vet. Med., 78(1):79-84.
26. MacFaddin, J.F. (1976): Biochemical tests for identification of medical bacteria .1-Ed., Williams and Wilkins Co., Baltimore, USA.
27. MacFaddin, J.F. (2002): Biochemical tests of identification of medical bacteria .3rd Ed., Lippincott Williams and Wilkins Washington, Philadelphina, USA.
28. Mohammed M.O. Amal A. Megahed and Saad S. Nasr. (2015): Bacteriological and molecular detection of Pseudomonas species from raw milk sold in Port-Said Citymarkets. Egypt. J. Chem. Environ. Health, 1 (1):986-1002.
29. Morgan, J. R., and Shelton IV, F. E. (2014): The synergistic effect of gentamycin and ceftazidime against *Ps. flourescence* U.S. Patent Application No. 14/298,252.
30. Muller, C.; Plesiat, P. and Jeannot, K. (2011): Two-component regulatory system interconnects resistance to polymyxins .Aminoglycosides, Fluoroquinolones and B-lactamase in *Ps. aeruginosa* . Antimicrobial Agents Chemotherapy.; 55 (3) 1211-1221.
31. Pfeifer, Y.; Cullik, A. and Witte, W. (2010): Resistance to Cephalosporin and carbapenemases in Gram-negative bacterial pathogens . Int. J. Med. Microbial .300:371-379.
32. Rizk-Heba (2014): Microbiological evaluation of some imported frozen meat, M.V.Sc, Thesis (Meat Hygiene), Benha University.
33. Roberts, R.J. (2001): "Fish pathology "3rd Ed .Bailliere Tindal, London England.
34. Schreckenberger, P.C. and Binnicker, M.J. (2011): Optimizing Antimicrobial susceptibility test reporting, 708: 15-19.
35. Serrano, P.H., (2005): Responsible use of antibiotics in aquaculture. FAO Fisheries Technical Paper, 469:3-9.
36. Settanni, L. and Corsetti, A. (2007): The use of multiplex PCR to detect and differentiate food &beverage-associated M .O: A review.J.and Microbial Meth. 69(1): 22-25.
37. Tauxe, M.A. (2002): Emerging food borne pathogens .Int.J.Food Microbial. 78(1-2):31-41.
38. Venugopal, V.; Pansare, a .c. and Levris, N.F. (1984): Inhibitory effect preservatives on protease secretion by Aermonas hydrophilia .Journal of Food Science; 49:1078-1081.
39. Washington, J.A (1981): Laboratory procedure in clinical Microbiology Springer .Verlag, New York Inc
40. WHO (2007) Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance?