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 10^3 to 1.9 x 10^7 with mean value 2.24 x 10^5 cfu/g. In addition, Psychrotrophic bacterial count in frozen minced meat ranged from 7 x 10^3 to 9 x 10^7 with mean value 1.7 x 10^6 cfu/g. The incidence of identified Pseudomonas species (number and percentages) detected in the examined samples of frozen meat represented by Ps. aeruginosa, Ps. fluorescenc, 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. fluorescenc, 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. fluorescenc. PCR is rapid and reliable tool for identification of different bacterial species.
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 et al., 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. Nitratreduction test (Kiss, 1984)
5. Indol production test (Bialy and Scott, 1978)
6. Methyl red test (Cruick Shank et al., 1975)
7. Voges – proskauer test (Cruick Shank et al., 1975)
8. Cytochrome oxidase test.
9. Growth at 42 ºc and 4 ºc (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 Diffusion disc method according to (Schreckenberger and Binnicker, 2011).

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.

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

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

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

Table 1 The used antimicrobial agents in the antibiotic sensitivity test

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

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

Results of antibiotic sensitivity tests against Ps. aeruginosa is demonstrated in table (6).
Ps. aeruginosa were sensitive to Gentamycin, Tobramycin, Cefepime, Cefazidime, 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 Cefazidime Gentamycin and Ciprofloxacin. Actually Ps. diminuta was more sensitive to Amikacin, Cefazidime 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, Cefazidime, Ciprofloxacin, Gentamycin, Imipenem, Ceftriaxone and Tobramycin.

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

The recorded virulence factors 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).

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 \times 10^2) to (9 \times 10^5) with mean value (1.7 \times 10^4). 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, (1.7 \times 10^3 \pm 2.1 \times 10^1) cfu/g from Alex Seaport, (9.9 \times 10^2 to 4.2 \times 10^6) with mean value (3.7 \times 10^5 \pm 7.9 \times 10^4) cfu/g and (9.9 \times 10^2 to 4.2 \times 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 \times 10^5 to 1.8 \times 10^5 with mean value 2.78 \times 10^2 to 2.18 \times 10^5 cfu/g, 1.7 \times 10^3 to 7.9 \times 10^3 with mean value 3.87 \times 10^2 to 4.35 \times 10^2 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. aeruginosa, Ps. fluorescence, Ps. diminuta, Ps. putida 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.

Table 5 Incidence of pseudomonas species isolated from examined samples of frozen 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 | 7 14 | 4 8 |
| Ps. fragi | 4 8 | 4 8 |

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

Table 6 Results of antibiotic sensitivity tests against Ps. aeruginosa

| Antimicrobial Agent | Fluorescenc | C. aeruginosa | P. putida | Ps. diminuta | Ps. fragi |
|-------------------|------------|--------------|-----------|-------------|----------|
| Amikacin (AK)     | R          | S            | R         | S           | R        |
| Cefepime (FEP)    | R          | S            | S         | R           | S        |
| Cefazidime (CAZ)  | R          | S            | S         | S           | R        |
| Ciprofloxacin (CIP) | R      | R            | S         | S           | R        |
| 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           | S        |

R: resistant, S: sensitive, I: intermediate
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, Cefazidime, 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 Cefazidime 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, Cefazidime, 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, Cefazidime 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. (Pfeffer 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. CONCLUSIONS

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.

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