

Investigation of the Incidence of *Pseudomonas sp.* in Foods

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Abstract

In this study, 100 different water and food samples collected from different sales point in district of İzmir, were examined for the presence of *Pseudomonas spp.*, in order to determine the incidence in foods. *Pseudomonas* species were isolated from 21 (7 minced beef, 11 milk, 2 chicken, 1 anchovy) of the samples and 8 of the isolates were identified as *P. aeruginosa*.

Key Words: *Pseudomonas sp.*, milk, and milk products, meat and meat products, vegetables, tap water.

INTRODUCTION

It is known that food is a cause of some diseases and has an important role for spreading these diseases. Food originated diseases can be depended on the factors of microbial, chemical, herbal and animal. The rate of distribution of these kinds of diseases can not be exactly defined due to the fact that food originated diseases are not informed health institutions, not all of them are diagnosed, and among the informed ones the highest rate is microbiological diseases.

When some microorganisms are balanced by normal microflora, under appropriate conditions (the worsening of immune system, chemotherapy and etc.) can become pathogen and these microorganisms are called opportunist pathogen [1]. For the opportunistic pathogen bacteria *Staphylococcus*

aureus, *Staphylococcus epidermidis*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, *Pseudomonas aureuginosa*, *Serratia marcescens*, *Listeria monocytogenes*, *Neisseria asteroides*, *Mycobacteria*, *Pediococcus acnes*, *Corynebacterium neoformans*, and for the opportunistic pathogen molds *Aspergillus sp.* and for the opportunistic pathogen yeasts *Candida albicans* and for the opportunistic pathogen viruses *Herpes simplex*, *Varicella zoster*, *Cytomegalovirus* are the good examples of them [2].

Pseudomonas kinds of bacteria including opportunist species and taking place in *Pseudomonaceae* family, produce various fluorescent and fluorescent non pigments, moving with polar flagella and gram negative bacteria [3].

In this study, *Pseudomonas spp.* analyse was made in some food and water. It is determined that the prevalence of *Pseudomonas* in food samples.

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MATERIALS AND METHODS

The material of this research include 17 water (100 ml), some vegetables (parsley, dill, lettuce, squash, tomato, radish, onion, celery, carrot, cabbage (100 g), also 19 raw milks and 8 cheese samples (100 g/ml) in portions. These samples were taken to the laboratory and started to be analysed within one hour at most.

Ten grams or ml of each sample were weighed under aseptic conditions and added to 90 ml under aseptic conditions and added to 90 ml of 0.1% ml (w/v) peptone water. To obtain aerobic plate counts (APCs) 1 ml of several ten fold dilution's were plated by the pour plate method using Plate Count Agar (Difco). The homogeneous dispersal and suspension in the medium was done by rotating the petri dishes . The plates were incubated 24-48 h at 30°C. To count psychrophilic bacteria Plate Count Agar was used and incubated at 5°C for 7 days [3].

Pseudomonas isolation was done attaching SR103 and 102 to *Pseudomonas* Agar Base [4]. After incubation, under U.V light (366 nm) colonies producing pigment. Suspected colonies producing white, yellow and green pigments as a result of incubation in *Pseudomonas* CFC agar and *Pseudomonas* CF agar during 24-48 h at 30°C have applied inoculation to EMB agar for *Pseudomonas* spp. identification. Uncolored colonies are lactose negative and blue-black and greenish bright dark colonies are lactose positive as a result of incubation during 48 h at 30°C. Among these, with the lactose negative ones are continued to be studied on and these colonies were applied oxidation-fermentation test. *Pseudomonas* react oxidative. Among the same samples catalase (+), motility (+) and oxidase (+) colonies are studied on [5].

For *Pseudomonas* identification, the features of

growth at 4°C and 41°C have been the first taken under consideration. So, *Pseudomonas*, which incubated into yeast extract medium were left at 4°C for 7-10 days and at 41°C for 24 h respectively [6].

In this study, *Pseudomonas* P (King A) and *Pseudomonas* F (King B), Cetrimide agar (CA) and Seller's Differential agar (SDA) medium were used for identification of *Pseudomonas* species isolated from different samples. Especially, *Pseudomonas* producing pyocyanin pigment and fluorescein pigment were evaluated King A and King B medium, respectively [7].

While some *Pseudomonas* species were producing very well, some were not producing in Cetrimide agar and Seller's Differential agar medium at all. As a result of incubation, nitrate denitrification was evaluated by observing gase production [8].

The above tests were made according to the literature [9-11].

RESULTS AND DISCUSSION

Total mesophilic bacteria and psychrophilic bacteria counts and isolation of *Pseudomonas* species have been made in the samples of raw milk, minced beef, water, cheese, chicken breast, chicken wing, anchovy, sardine, onion carrot, cabbage, parsley, dill and celery taken by various sale points in Izmir.

Table 1. *Pseudomonas* species found in our study and the name of food.

<i>Pseudomonas</i> species	Milk	Minced beef	Chicken	Fish	Natural water	Raw vegetables	Cheese
<i>P. aeruginosa</i>	4	1	1	0	0	0	0
<i>P. cepacia</i>	4	0	0	0	0	0	0
<i>P. fluorescens I</i>	0	4	0	0	0	0	0
<i>P. fluorescens II</i>	3	1	0	0	0	0	0
<i>P. fluorescens III</i>	0	3	1	1	0	0	0
<i>P. putida</i>	0	1	0	0	0	0	0

Among the examined samples in 11 out of 19 raw milk, 10 out of 16 minced beef, 2 of 11 chicken breasts and 1 out of 6 fish *Pseudomonas* species were isolated. The results were given in Table 1.

The Samples of Milk and Cheese

In the 19 samples of raw milk and 9 samples of cheese taken by various sale points in Izmir. *Pseudomonas* species have been looked for. Besides this psychrophilic and mesophilic aerobic counts have been in order to determine the microbial quality of raw milk.

The average number of mesophilic bacteria counts searched on 19 raw milk has been found 3.1×10^7 cfu/ml and the average number of psychrophilic bacteria counts 2.9×10^5 cfu/ml in our research.

In different studies, the existence of *P. aeruginosa* in raw milk is changing between 4% and 27% [12-17]. The results taken in this study are similar to the ones by Cheung and Westhoff (33.3%)

The existence of different studies *P. cepacia* is changing between 1.1% and 12% % [13-16]. The results taken in this study are similar to the ones by King (33.3%).

The existence of *P. fluorescens* in raw milk in various studies is differing from 7% and 83% [12-14, 18-20]. The result of this study is similar to which was reported by Milliere and Veilet –Pancet (33.3%) [19].

While in the studies the ratio of *Pseudomonas* was given in psychrophilic flora, in our study the ratio of *Pseudomonas* species was given according to the finding *Pseudomonas*. So, this causes some differences. Other studies were made one or two years, but our raw milk samples were examined in summer season.

The samples of Chicken, Minced Beef and Fish

11 chicken (chicken breast and chicken wing), 16 minced beef, 6 fish (3 sardalya and 3 anchovy) samples were taken from various sale points in Izmir and *Pseudomonas* species have been investigated. Also psychrophilic and mesophilic aerobic counts have been taken in order to determine the microbial quality of raw milk.

The average number of mesophilic bacteria counts searched on 11 chicken has been found 1.6×10^5 cfu/g and the average number of psychrophilic bacteria counts 1.5×10^4 cfu/ml in our research. The average number of mesophilic bacteria counts searched on 16 minced beef has been found 3.1×10^6 cfu/g and the average number of psychrophilic bacteria counts 3.2×10^5 cfu/g. The average number of mesophilic bacteria counts searched on 3 anchovy has been found 1.8×10^4 cfu/g the average number of psychrophilic bacteria counts 2.4×10^3 cfu/g and also the average number of mesophilic bacteria counts searched on 3 sardalya has been found 2.6×10^5 cfu/g, the average number of psychrophilic bacteria counts 5.3×10^4 cfu/g.

In a study, *P. aeruginosa* ratio of meat was found 2.3 % [21]. In our study the *P.aeruginosa* ratio was 13.3%.

The results of the study were taken in summer season. According to Uraz and Çıtak, isolation of *Pseudomonas* were increasing in summer season [22].

In various studies *P. putida* ratio of meat is differing from 6-50 % [23, 24]. The results taken at this study, were similar to the ones by Rollier et al (20%) [21].

In different research, the ratio of *P. fluorescens* has been found between 5% and %80 [23-27]. On the other hand in our study, the existence of *P.*

fluorescens biotype I, *P. fluorescens* biotype II, *P. fluorescens* biotype III were found 26.7, 13.3 and 26.7% respectively. The results taken at this study, were similar to the ones by Sundheim and his colleagues [27].

The Samples of Vegetable

In the natural flora of vegetables especially the species of *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter*, *Leuconostoc*, *Serratia*, *Lactobacillus*, *Enterobacter*, *Micrococcus*, *Serratia* and *Streptococcus* and as plant pathogen *Erwinia* and *Xanthomonas* species [27].

Pseudomonads cause root softness especially in potatoes, carrots, celery. The most important feature of the organism results in softening the plant tissue by secreting enzyme [28].

The studies on the organisms growing in plant roots and the reason for ready-made salad's spoiling caused by *Pseudomonas* species have been found in fresh raw vegetables. Wrigt and his colleagues found *P. aeruginosa* in the 44% of 114 vegetable salads in hospital [29].

Brocklehurst and his colleagues (1987) kept vegetable salads at 7°C by the expiration date and found that *Pseudomonas sp.* and *Enterobacter agglomerans* were prevalent [30].

In these studies, *Pseudomonads* was looked for especially kept samples and softened. But in our study the samples were fresh and edible. For this reason no *Pseudomonas* were found.

The Species of *Pseudomonas* in natural water

The *Pseudomonas* species were looked for in the 17 samples of natural water taken from different hospitals and parks in Izmir.

In none of the samples studied on *Pseudomonas* species were found. This is resulted from the amount of chlorine used in natural water destroying all microorganisms.

In different studies, while Jayasekara and his colleagues (1998), were found dominant flora 64% *Pseudomonas* [31], Bharath and his colleagues were found 7.6% *Pseudomonas* [32].

In conclusion, we determined that *Pseudomonas* species were found in especially milk and meat samples due to proteolytic and lypolytic. No *pseudomonas* species found in water and vegetables samples.

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