

# Investigation of The Incidence of *Pseudomonas aeruginosa* in Foods and The effect of salt and pH on *P. aeruginosa*

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## Abstract

In this study, 100 different water and foods samples, collected from different sales point in district of İzmir, were examined for the presence of *Pseudomonas aeruginosa* in order to determine the incidence in foods. *Pseudomonas aeruginosa* was isolated from milk and meat samples and 8 of the isolates were identified as *P. aeruginosa*. As for as effect of salt concentration and at pH growth on *P. aeruginosa*. It was not grow at 7% salt concentration and pH 5.

**Keywords:** *Pseudomonas aeruginosa*, milk, and milk products, meat and meat products, vegetables, tap water, pH, salt concentration.

## 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, chemotraphy and etc.) can become pathogen and these microorganisms are called opportunist pathogen [4]. 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 patogen molds *Aspergillus spp.* and for the opportunistic patogen yeasts *Candida albicans* and for the opportunistic patogen viruses *Herpes simplex*, *Varicella zoster*, *Cytomegalo* are the good examples of these [20].

*Pseudomonads* 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 [4].

In this study *Pseudomonas aeruginosa* analyse was made in some food and water. It is determined that prevalence of *Pseudomonas aeruginosa* in food samples and the effect of salt and pH on *P.aeruginosa*.

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## MATERIALS AND METHODS

The material of this research include 17 water (100ml), some vegetables (parsley, dill, lettuce, squash, tomato, radish, onion, celery, carrot, cabbage (100gr), also 19 raw milks and 8 cheese samples (100gr/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) 1ml 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 hours at 30°C. To count psychrophilic bacteria Plate Count Agar was used and incubated 5°C for 7 days [24].

*Pseudomonas* isolation was done attaching SR103 and 102 to *Pseudomonas* Agar Base. After incubation, under U.V light (366nm) 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 hours 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 hours 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 [16].

For *Pseudomonas* identification, the features of

growth at 4°C and 41°C have been the first taken into consideration. So, *Pseudomonas*, which incubated into yeast extract medium were left at 4°C for 7-10 days and at 41°C for 24 hours respectively [18].

In this study, *Pseudomonas* P(King A) and *Pseudomonas* F(KingB), 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 [23].

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

The following tests were made with reference to the Shaw and Laty [22] and Koneman et al [15].

### The effect of salt and pH on *P. aeruginosa*

*P. aeruginosa* ATCC 27853 was used for as a culture. 10 ml TSYEB medium containing different salt consantrations. The percentage of 3, 5, 7 % salt consantrations were used in this study. Also no salt containing TSYEB medium were used as a control. The four different inoculum doses of *P. aeruginosa* were inoculated into TSYEB medium.

The same method used for effect of pH 5, 6, 7, 8 levels were used in this study. As a control used for pH 7.3 due to TSYEB medium' s pH 7.3. Four different inoculum doses of *P. aeruginosa* has been inoculated into TSYEB medium with different pH levels. After incubation 48 hours at 35-37°C, inoculated into TSYEA and colonies have been numbered.

## RESULTS AND DISCUSSION

Total mesophilic bacteria and psychrophilic bacteria counts and isolation of *Pseudomonas aeruginosa* has 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.

### 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  $3,1 \times 10^7$  cfu/ml and the average number of psychrophilic bacteria counts  $2,9 \times 10^5$  cfu/ml in our research.

In different studies, the existence of *P. aeruginosa* in raw milk is changing between 4% and 27%. [7, 11-13, 17, 25]. The results taken in this study are similar to the ones by Cheung and Westhoff (33,3%).

### The samples of Chicken, Minced Beef and Fish

In the 11 chicken (chicken breast and chicken wing), 16 minced beef, 6 fish (3 sardines and 3 anchovies) samples of 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 11 chicken has been found  $1.6 \times 10^5$  cfu/g and the average number of psychrophilic bacteria

counts  $1.5 \times 10^4$  cfu/ml in our research. The average number of mesophilic bacteria counts searched on 16 minced beef has been found  $3.1 \times 10^6$  cfu/g and the average number of psychrophilic bacteria counts  $3.2 \times 10^5$  cfu/g. The average number of mesophilic bacteria counts searched on 3 anchovies has been found  $1.8 \times 10^4$  cfu/g. The average number of psychrophilic bacteria counts  $2.4 \times 10^3$  cfu/g and also the average number of mesophilic bacteria counts searched on 3 sardines has been found  $2.6 \times 10^5$  cfu/g. The average number of psychrophilic bacteria counts  $5.3 \times 10^4$  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 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 [1].

*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 [18].

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

Wright and his colleagues [27] found *P. aeruginosa* in the 44% of 114 vegetable salads in hospital. Brocklehurst and his colleagues [6] kept vegetable salads at 7°C by the expiration date and found that *Pseudomonas spp.* and *Enterobacter agglomerans* were prevalent.

In the 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* was 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 were found dominant flora 64 % *Pseudomonas*, Bharath and his colleagues were found 7.6% *Pseudomonas*.

### The effect of salt on *P. aeruginosa*

It was given Table 1, the effect of salt on *P.aeruginosa*. While 7% salt concentration was bacterisidal effect on *P. aeruginosa*, 5% salt concentration was bacteriostatic in all doses. Bacterisidal effect appeared in all doses in which 7% salt concentration was used. While 5% salt concentration caused bacterisidal effect on  $2.2 \times 10^3$  and 15 kob/ml, inoculum doses, bacteriostatic effect

was observed in other doses.

No salt tolerance in *P. aeruginosa* hadn't been come up with, so it was studied considering minimum salt concentrations. When we looked at the rate of salt changes in food, like minced beef, dehydrated cabbage pickle, olive, cheese in these percentages 3%, %2.25-2.5, %6-9, %1-1.5 respectively [1].

The salt in cabbage pickle, existent lactic acid bacteria in food were caused to be dominant by inhibiting bacteria resulted in spoilt normal flora like *Pseudomonas*. After our study, no *P. aeruginosa* resistance to salt was determined. While *P. aeruginosa* was resistant to 3% salt concentrations, it was inhibiting or dying. In higher salt concentrations, it was inhibited or dead.

### The effect of pH on *P. aeruginosa*

The effect of pH on *P. aeruginosa* growth in our study of four different inoculum doses was given Table 2. The cell number of *P. aeruginosa* increased to the level of  $10^8$ - $10^9$  As a result of the incubation for 48 hours in all doses of inoculum TSYEB medium as seen on the table above while pH 5 had a bactericidal effect on all inoculum doses and pH 6 had the same effect on minimum inoculum dose, in

Table 1. The effect of salt on *P. aeruginosa* in laboratory medium (37°C/ 48 hours)

		Inoculum	dose	cell/ml		
			$1.5 \times 10^1$	$2.2 \times 10^3$	$4.2 \times 10^5$	$3.0 \times 10^7$
<i>P. aeruginosa</i>						
Salt (%)	pH					
Control	7.3	$1.8 \times 10^8$	$2.6 \times 10^9$	$7.8 \times 10^9$	$4.2 \times 10^9$	
3	7.4	$3.0 \times 10^1$	$4.0 \times 10^3$	$5.0 \times 10^5$	$3.6 \times 10^7$	
5	7.45	0	0	$3.6 \times 10^1$	$2.0 \times 10^2$	
7	7.55	0	0	0	0	

Table 2. The effect of pH on *P. aeruginosa* in laboratory medium (37°C/ 48 hours)

	Inoculum	dose	cell/ml	
	1.5x10 <sup>1</sup>	2.2x10 <sup>3</sup>	4.2x10 <sup>5</sup>	3.0x10 <sup>7</sup>
<i>P. aeruginosa</i>				
pH				
5	0	0	0	0
6	0	2.1x10 <sup>3</sup>	4.5x10 <sup>7</sup>	2.1x10 <sup>8</sup>
7	3.2x10 <sup>9</sup>	4.6x10 <sup>9</sup>	3.3x10 <sup>9</sup>	6.5x10 <sup>9</sup>
8	0	0	2.6x10 <sup>2</sup>	2.2x10 <sup>4</sup>

maximum inoculum doses, it had a bacteriostatic effect. Bacteriosidal effect had a role in minimum inoculum dose at pH 8. The high incubation dose had been getting 3 log less than the dose at the beginning, but it didn't have a bacteriosidal effect.

In conclusion, we determined that *Pseudomonas aeruginosa* were found in especially milk and meat samples due to proteolytic and lipolytic. There has not any relationship between incidence of *P. aeruginosa* and count of mesophilic aerobic and psychrophilic bacteria. No *Pseudomonas aeruginosa* found in water and vegetables samples. Salt concentration of 5% higher than is bacteriostatic or bacteriocidal effect on medium according to inoculum doses and same effects were observed.

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