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Effects of different temperatures on virulence factors and antimicrobial resistance of *Aeromonas* isolated from retail food

Einfluss unterschiedlicher Temperaturen auf die Virulenzfaktoren und die antimikrobielle Resistenz von Aeromonas Isolaten aus Lebensmitteln

Ergin Kariptas[1], Belgin Erdem[1], Sener Tulumoglu[2]

Summary

The aim of this study was to evaluate the public health significance of representative motile *Aeromonas* isolates, which were mainly isolated from different foods, and to determine the effects of different temperatures on virulence factors such as hemolysin, protease, lipase and nuclease. The influence of different temperatures (+4 °C, +25 °C and +37 °C) on virulence factors of 40 isolates of *Aeromonas (A.)* spp. (*A. hydrophila, A. caviae* and *A. sobria*) was investigated. All isolates could produce hemolysin, protease, lipase, and nuclease at +37 °C, +25 °C and +4 °C; however, these virulence associated factors were produced faster at +37 °C and +25 °C than at +4 °C. All *Aeromonas* species were also tested for antibiotic resistance patterns and were found to be resistant to ampicillin, yet sensitive to imipenem, ciprofloxacin and amikacin antibiotics.

Keywords: *Aeromonas,* virulence, temperatures, antimicrobial resistance

Zusammenfassung

Ziel dieser Studie war es, die Bedeutung von repräsentativen beweglichen *Aeromonas* Isolaten, welche hauptsächlich aus verschiedenen Lebensmitteln stammten wurden, für die öffentliche Gesundheit zu beurteilen, sowie den Einfluss verschiedener Temperaturen auf Virulenzfaktoren, wie Hämolysin, Protease, Lipase und Nuklease, zu ermitteln. Der Einfluß verschiedener Temperaturen (+4 °C, +25 °C und +37 °C) auf die Virulenzfaktoren wurde bei 40 *Aeromonas (A.)* spp. Isolaten untersucht (*A. hydrophila, A. caviae* und *A. sobria*). Alle Isolate produzierten Hämolysin, Protease, Lipase und Nuklease bei +37 °C, +25 °C und +4 °C; jedoch wurden diese Virulenzfaktoren bei +37 °C und +25 °C schneller exprimiert als bei +4 °C. Alle *Aeromonas* Spezies wurden auch auf Antibiotikaresistenzen untersucht, wobei sie als resistent gegenüber Ampicillin, jedoch empfindlich für Imipenem, Ciprofloxacin und Amikacin ermittelt wurden.

Schlüsselwörter: *Aeromonas,* Virulenz, Temperaturen, antimikrobielle Resistenz

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Introduction

Bacteria of the genus *Aeromonas* have been frequently recognized as responsible for several diseases, both in humans and animals (González-Serrano et al., 2002; Wu et al., 2007). *Aeromonas (A.) hydrophila, A. caviae* and *A. sobria* have been linked to two groups of human diseases: septicemia and gastroenteritis (Vila et al., 2002; Radu et al., 2003; Hatha et al., 2005; Palu et al., 2006; Gunsalam et al., 2006). Food of animal origin, seafood and water have been considered important vehicles of *Aeromonas* spp. infections (Ullmann et al., 2005; Sharma et al., 2005; Ottaviani et al., 2006; Oliveira Scoaris et al., 2008). Several investigations have shown that members of the genus *Aeromonas* are also widely distributed in various foods such as meat (Neyts et al., 2000; Radu et al., 2003), fish (González-Serrano et al., 2002; Castro-Escarpulli et al., 2003; Hatha et al., 2005; Farag, 2006; Erdem et al., 2008) and chicken (McMahon, 2000).

Singh et al. (2000) reported that *A. hydrophila* were isolated from samples of water, soil and sediments from Leh (India) and showed enzyme activity at +20 °C and +37 °C. All isolates indicated production of protease, amylase and lipase simultaneously. A study (Sechi et al., 2002) carried out in Sardinia (Italy) showed a greater prevalence of hemolysin and protease production at +30 °C, among 46 isolates of different *Aeromonas* spp. strains isolated from patients with diarrhea and from coastal water. A number of putative virulence factors (aerolysin/hemolysin, proteases, lipases, DNases) that may play an important role in the development of disease, either in humans or in fish, have been described in several species of the genus (Soler et al., 2002).

Temperature-dependent differences for virulence factors (hemolysin, protease, lipase, nuclease, cytotoxin and enterotoxin) that may play an important role in the development of disease, pathogenicity and spoilage potential, either in humans or in fish, have been described in *A. hydrophila, A. caviae* (Martins et al., 2002) and *A. sobria* (Filler et al., 2000) as well as in the genus in general (Braun et al., 2001; Sechi et al., 2002; Soler et al., 2002). McMahon et al. (2000) postulated a correlation between the pathogenic potential and the hemolytic and proteolytic activity of *Aeromonas* species isolated from different sources. Motile aeromonads are considered as emerging food-borne pathogens because it was shown that some *Aeromonas* food isolates can produce different virulence factors, not only at optimal growth temperatures, but also at refrigeration temperatures (Castro-Escarpulli et al., 2003).

There has been only a limited amount of investigation on the effect of different temperatures on virulence factors of *Aeromonas* isolates from different foods (González-Serrano et al., 2002; Castro-Escarpulli et al., 2003; Ullmann et al., 2005; Oliveira Scoaris et al., 2008). Therefore, the present study was undertaken to examine *Aeromonas* isolated from foods, to evaluate the effect of different temperatures on virulence factors such as hemolysin, protease, lipase and nuclease. We also determined the resistance of the *Aeromonas* isolates to different antibiotics.

Material and Methods

Isolation and identification of *Aeromonas* species

Fifty samples of raw calf meat, 50 samples of chicken carcasses and 80 minced meat samples were collected from randomly selected local retail shops and supermarkets in Kırşehir (Turkey) for a one-year period. Foods were purchased in regular consumer packages and immediately transferred to the laboratory for analysis. Approximately 20 g of meat was aseptically added to 180 ml of alkaline peptone water (APW) containing 30 µg/ml of ampicillin (Sigma Chemical Co., St. Louis, Mo., USA) (APW, pH: 8.4) in a sterile stomacher plastic bag and homogenized for 2 min in a Colworth Stomacher 400. The APW was prepared as recommended by Gobat and Jemmi (1993). After all isolates were incubated at +28 °C for 24 h, a loopful of enrichment broth was streaked on glutamate starch phenol red agar (GSP agar, Merck, Darmstadt, Germany). The genus *Aeromonas* was identified based on the findings of positive oxidase test, fermentation of d-glucose, motility, the absence of growth in 6.5 % sodium chloride, and susceptibility to the vibriostatic agent O/129 (150 µg), as described previously (Altwegg et al., 1990). Identity was confirmed by the API 20E system (bioMérieux, Marcy-I'Etoile, France). *Aeromonas* species were further differentiated phenotypically by biochemical tests into the following three major groups: *A. hydrophila, A. sobria* and *A. caviae.* The schemes of biochemical identification proposed by Abbott et al. (2003) were followed. Tests for species identification included detection of ß-hemolysis, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, bile esculin hydrolysis, gas formation and D-glucose fermentation on TSI slant, indole test, Voges-Proskauer test, acid production from glucose-1-phosphate, rhamnose, lactose, sucrose, d-sorbitol, D-mannitol and salicine, and citrate utilization (Altwegg et al., 1990; Abbott et al., 2003). The isolates were stored at –70 °C in 15 % glycerol until required.

Strain collection

To control identification, the following American Type Culture Collection (ATCC®) strains were used: *A. hydrophila* ATCC® 7966, *A. caviae* ATCC® 15468, *A. sobria* ATCC® 43979.

Hemolytic, protease, lipase and nuclease activities

Hemolysin was determined using blood agar medium containing 5 % sheep blood collected aseptically. Beta hemolytic activity was recorded as clear zones around the colony after incubation at +37 °C and +25 °C for 2 d and +4 °C for 7 d. The hemolytic activity of the *Aeromonas* spp. isolates was categorized as alpha or beta (Gerhardt et al., 1981).

Protease was determined on the surface of skim milk agar in which skim milk was added just before pouring the medium into the petri plates. The plates were incubated at +37 °C, +25 °C and +4 °C for 7 d. After the incubation period, the clear zones of hydrolysis were measured and recorded. The presence of a transparent zone around the colonies indicated protease activity (Gudmundsdóttir, 1996).

Lipase was evaluated on the surface of tributyrin agar plates. The plates were incubated at +37 °C, +25 °C and +4 °C for 7 d, the medium appeared opaque but lipolytic colonies were surrounded by a clear zone (Koburger and Jacger, 1987).

Extracellular nuclease (DNase) was determined on DNase agar plates with 0.005 % methyl green. Five microlitres of each suspension was streaked onto the plates and incubated at +37 °C, +25 °C for 3 d and at +4 °C for 7 d. A pink halo around the colonies indicated nuclease activity. After incubation, the diameters of the colonies and the

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precipitation zones were measured (Edberg et al., 1996; Oliveira Scoaris et al., 2008).

Antimicrobial resistance

The resistance of all isolates to different antimicrobial agents was determined by the standard disc diffusion method of the National Committee for Clinical Laboratory Standards (NCCLS, 2003, 2004). The antibiotics and concentration ranges tested were as follows: amikacin (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), imipenem (10 µg), piperacillin (100 µg), tetracycline (30 µg), ceptazidime (30 µg), amoxicillin-clavulanic acid (30 µg), aztreonam (30 µg), moxifloxacin (5 µg) and ceptriaxone (30 µg).

The resistance breakpoints were those defined by NCCLS (2004) for Gram-negative bacteria. *Escherichia coli* ATCC® 25922, *Pseudomonas aeruginosa* ATCC® 27853, *A. hydrophila* ATCC® 7966, *A. caviae* ATCC® 15468 and *A. sobria* ATCC® 43979 were used as controls.

Statistical analysis

In this study Levene's test for homogenity and analysis of variance (ANOVA) were performed using SPSS 13.0 software to test the effects of different temperatures on virulence factors (hemolysin, proteinase, lipase and nuclease). P values less than 0.01 were considered to be statistically significant. Differences between averages were analyzed by using Duncan's multiple comparison test.

Results and Discussion

Detection of *Aeromonas* from food samples

The *Aeromonas* isolates used in this study and their source of isolation are listed in Table 1. *Aeromonas* species were isolated from 22.2 % (40/180) of the 180 food samples analysed. More specifically, they were isolated from 20 %, 10 % and 31.2 % of raw chicken, red meat and minced meat samples, respectively. Of the 40 isolates obtained, *A. hydrophila* was the most frequently isolated species (50 %), followed by *A. caviae* (30 %) and *A. sobria* (20 %). Generally, the studies on the prevalence of *Aeromonas* spp. in samples of environmental, clinical and food origin focused on the three species *A. hydrophila, A. sobria* and *A. caviae.* Several studies showed pronounced variations in their occurrence (Singh et al., 2000; Villari et al., 2000; Martins et al., 2002; Ullmann et al., 2005; Oliveira Scoaris et al., 2008). This observation is substantiated by our findings.

Raw and processed food is transported and kept at low temperatures for conservation (Mano et al., 2000; Ullmann et al., 2005). Therefore, we were interested to examine in our study if different (especially low) temperatures inhibit the growth of *Aeromonas* isolated from food. Other researchers have also reported the secretion of hemolysin (González-Serrano et al., 2002; Ullmann et al., 2005), protease (McMahon, 2000), lipase (Singh et al., 2000; El-Diasty and Salem, 2007) and nuclease (Castro-Escarpulli et al., 2003) from *Aeromonas.* Castro-Escarpulli et al. (2003) revealed that these factors were all common in *Aeromonas* strains, as 90 % showed aerolysin/hemolysin, 100 % lipase and 100 % DNase activity at +22 °C and +37 °C. McMahon et al. (2000) reported that *A. hydrophila* express extracellular proteinases and hemolysins in modified atmospheres containing low and moderate CO_2 concentrations at +28 °C, +10 °C and +5 °C.

Expression of hemolysin

The test results are shown in Table 2, the sheep blood agar plates were incubated at +37 °C and +25 °C for 2 d and at

TABLE 1: *Aeromonas spp. isolated from different foods.*

Type of sample	Number of samples		*Aeromonas* spp.		
	Analyzed	Positive (%)	*A. hydrophila*	*A. caviae*	*A. sobria*
Raw chicken	50	10 (20)	5 (50)	3 (30)	2 (20)
Red meat	50	5 (10)	2 (40)	2 (40)	1 (20)
Minced meat	80	25 (31.25)	13 (52)	7 (28)	5 (20)
Total	180	40 (22.20)	20 (50)	12 (30)	8 (20)

TABLE 2: *Production of hemolysin, proteinase, lipase and nuclease at growth temperatures of +37 °C, +25 °C and +4 °C.*

Species	Numbers of isolates (n)	Isolates producing hemolysin [n (%)]				Isolates producing proteinase [n (%)]			
		+37 °C	+25 °C	+4 °C	Mean	+37 °C	+25 °C	+4 °C	Mean
A. hydrophila	20	20 100±0.00	20 100±0.00	16 80.00±2.88	93.33±3.43[a]	20 100±0.00	20 100±0.00	16 80.00±2.88	93.33±3.43[b]
A. caviae	12	0 0±0.00	0 0±0.00	0 0±0.00	0±0.00[b]	8 63.83±2.76	8 63.83±2.76	6 49.96±4.82	59.21±2.92[a]
A. sobria	8	8 100±0.00	8 100±0.00	6 75.00±7.21	91.66±4.65[a]	8 100±0.00	8 100±0.00	6 75.00±0.00	91.66±4.16[b]
Mean (%)		66.66±16.66[b]	66.66±16.66[b]	51.66±13.12[a]		87.94±6.08[b]	87.94±6.08[b]	68.32±4.92[a]	

Species	Numbers of isolates (n)	Isolates producing lipase [n (%)]				Isolates producing nuclease [n (%)]			
		+37 °C	+25 °C	+4 °C	Mean	+37 °C	+25 °C	+4 °C	Mean
A. hydrophila	20	20 100±0.00	20 100±0.00	8 40.00±2.88	80.00±9.91[c]	20 100±0.00	20 100±0.00	16 80.00±2.88	93.33±3.43[b]
A. caviae	12	4 33.30±0.00	4 33.30±0.00	2 16.60±0.00	27.73±2.78[a]	8 66.66±5.47	8 66.66±2.63	6 50.00±8.63	61.10±3.21[a]
A. sobria	8	5 62.50±0.00	5 62.50±0.00	4 50.00±7.21	58.33±2.94[b]	8 100±0.00	8 100±0.00	6 75.00±0.00	91.66±4.16[b]
Mean (%)		65.26±9.65[b]	65.26±9.65[b]	35.53±5.43[a]		88.88±5.23[b]	88.88±5.12[b]	68.33±3.56[a]	

[a], [b], [c]:: Differences between means indicated by different superscript letters in the same line and column are significant (P<0.01).

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Archiv für Lebensmittelhygiene 61, Heft 2 (2010), Seiten 37–80

+4 °C for 7 d after inoculation with bacteria. Among the *Aeromonas* isolates obtained in the present study, 20 *A. hydrophila* (100 %) and eight *A. sobria* (100 %) showed hemolysin activity at +37 °C and +25 °C. Sixteen (80 %) of the 20 *A. hydrophila* and six (75 %) of the eight *A. sobria* isolates expressed hemolysin at +4 °C. In contrast, none of the *A. caviae* isolates showed hemolysin activity at any of the incubation temperatures (Tab. 2). Beta hemolysin has been reported as a virulence factor in motile aeromonads (Martins et al., 2002; Radu et al., 2003).

The present study has demonstrated that *A. hydrophila* and *A. sobria* produce hemolysin, whereas *A. caviae* are nonhemolytic. A relationship between hemolysin production at different temperatures (+37 °C, +25 °C, +4 °C) and the isolates of *A. hydrophila* and *A. sobria* was significant ($p<0.01$), but the relationship between *A. caviae* and hemolysin production at these temperatures was not significant ($p>0.05$). These results agreed with the results recorded by Farag (2006) who showed a significant difference between the hemolysin production of *A. hydrophila* and *A. sobria* compared with *A. caviae.*

A study of Ullmann et al. (2005) on 84 seafood samples which were purchased from retail traders in Berlin showed that hemolytic activity was detected in 98.5 % of the 134 *Aeromonas* isolates at +28 °C and in 97.0 % of the isolates at +37 °C incubation. More than 90 % of the *A. hydrophila* produced hemolysins at +4 °C. In contrast, ten (41.7 %) of the *A. caviae* and none of the *A. sobria* isolates showed hemolysis at +4 °C (Ullmann et al., 2005). Similarly, a study by Palu et al. (2006) revealed that 100 % of *A. hydrophila,* 50 % of *A. sobria* and 77.8 % of *A. caviae* exhibited beta hemolytic activity at +37 °C. Our findings are consistent with the observations of Ullmann et al. (2005) for *A. hydrophila,* but in contrast concerning *A. sobria* and *A. caviae.*

The results suggested that potentially human pathogenic *A. hydrophila* and *A. sobria* strains are present in raw food indicating a food safety problem.

Expression of protease

Our findings demonstrated that protease was highly prevalent in the isolates at +37 °C, +25 °C and +4 °C. In this study, 100 % of the *A. hydrophila* isolated from food were producers of protease at +37 °C and +25 °C as well as 100 % of the *A. sobria* and 63.8 % of the *A. caviae* isolates. As can be seen in Table 2, protease activity was detected in 16 (80 %) of 20 *A. hydrophila,* six (75 %) of eight *A. sobria* and six (49.9 %) of twelve *A. caviae* isolates from food at +4 °C. González-Serrano et al. (2002) have found similar results among *A. hydrophila* and *A. veronii* biovar sobria. The isolates produced variable amounts of proteases at different temperatures (+37 °C, +28 °C and +4 °C).

Proteases are important factors in the spoilage of foods, and the presence of proteases and hemolysins is used as an indicator of potential pathogenicity (McMahon, 2000). Oliveira Scoaris et al. (2008) reported that both the quantitative and qualitative determination of protease is important in establishing the virulence of a particular strain. Finally, our findings revealed that proteases more than hemolysin may be important virulence factors in *Aeromonas* infections. Proteases are thought to contribute to the virulence of aeromonads for fish and other hosts, however, their contribution to human pathogenicity still needs to be determined.

Expression of lipase

Lipase activity was detected in 20 (100 %) of 20 *A. hydrophila,* five (62.5 %) of eight *A. sobria* and four (33.3 %) of twelve *A. caviae* isolates at +37 °C and +25 °C. Additionally, it was observed in eight (40 %) of 20 *A. hydrophila,* four (50 %) of eight *A. sobria* and two (16.6 %) of twelve *A. caviae* isolates at +4 °C. It is clear that *A. caviae* was the species with the least presence of lipase activity at +4 °C. The results of the present study indicate a difference in the expression of lipase at different temperatures in *Aeromonas* isolates. These results are similar to those reported by Braun et al. (2001) who observed that all lipases of *A. hydrophila* and *A. caviae* strains digest tributyrin at +7 °C. The members of this genus may have the same pattern of increasing synthesis of lipase at high temperature and decreasing production below +4 °C thus showing higher activity at +37 °C and +25 °C than at +4 °C. Our findings were consistent with the results of several other studies (Singh et al., 2000; Braun et al., 2001; Castro-Escarpulli et al., 2003; Adham, 2003).

The influence of endogenous lipases in food during ripening or spoilage processes has been frequently described (Driessen and Stadhouders, 1975; Law, 1979; Vlaemynck, 1992). It is well known that lipases are able to hydrolyse fat at different temperatures. Little is known about the amount of bacterial lipases in different food. So, it is suggested that lipases can be one of several factors determining pathogenicity but are not required for virulence in all *Aeromonas* species.

Expression of nuclease

Nuclease (DNase) activity was detected in 20 (100 %) of 20 *A. hydrophila,* eight (100 %) of eight *A. sobria* and eight (66.6 %) of twelve *A. caviae* isolates at +37 °C and +25 °C. DNase activity also was detected in 16 (80 %) of 20 *A. hydrophila,* six (75 %) of eight *A. sobria* and six (50 %) of twelve *A. caviae* isolates at +4 °C (Tab. 2). DNases have also been considered as possible nutritional enzymes (Oliveira Scoaris et al., 2008). One of the extracellular enzymes produced by *Aeromonas* which is DNase has been identified by several researchers (Soler et al., 2002; Oliveira Scoaris et al., 2008). The results obtained in this study were nearly similar to those reported by Castro-Escarpulli et al. (2003), who found that 90 % DNase activity was present in *Aeromonas* isolates at +22 °C and +37 °C. Recently, a similar type of work has also been reported by Oliveira Scoaris et al. (2008).

Our results indicated that the effect of different temperatures on the DNase production can be clearly seen. It is notable that *Aeromonas* isolates produced considerable DNase activities at different temperatures (+37 °C, +25 °C and +4 °C). This information might be useful for researches with respect to DNase activity and pathogenicity in *Aeromonas.*

Statistically, using analysis of variance (ANOVA), it was revealed that the differences between the growth temperatures in terms of production of hemolysin, protease, lipase and nuclease (Tab. 2) were significant ($P<0.01$). Most of *Aeromonas* isolates produced detectable hemolysin, protease, lipase and nuclease at all tested temperatures (+37 °C, +25 °C and +4 °C). In most cases, *Aeromonas* expressed higher hemolysin, proteinase, lipase and nuclease production at +37 °C and +25 °C than at +4 °C ($P<0.01$).

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Antibiotic resistance

The percentage of *Aeromonas* spp. showing resistance against each antibiotic included in the study is given in Table 3. In total, 100 % of the isolates (*A. hydrophila, A. caviae* and *A. sobria*) showed resistance to moxifloxacin and ampicillin while 75 % were resistant to amoxicillin-clavulanic acid, 65 % to piperacillin, 60 % to aztreonam, 30 % to ceptazidime and 15 % to tetracycline. On the contrary, 100 % of the isolates were susceptible to imipenem, ciprofloxacin, ceptriaxone and amikacin. *Aeromonas* isolates from seafood in Mexico and Malaysia (Castro-Escarpulli et al., 2003; Radu et al., 2003) showed resistance to imipenem while we encountered no resistance to this antibiotic.

According to our results, 14 (70 %), 13 (65 %), twelve (60 %), five (25 %) and three (15 %) of 20 *A. hydrophila* isolates yielded resistance to amoxicillin-clavulanic acid, piperacillin, aztreonam, ceptazidime and tetracycline, respectively. Of twelve *A. caviae* isolates, ten (83.3 %), nine (75 %), seven (58.3), four (33.3 %) and two (16.6) showed resistance to piperacillin, amoxicillin-clavulanic acid, aztreonam, ceptazidime and tetracycline, respectively. Of eight *A. sobria* isolates, seven (87.5 %), five (62.5 %), three (37.5 %), three (37.5 %) and one (12.5 %) were resistant to amoxicillin-clavulanic acid, aztreonam, piperacillin, ceptazidime and tetracycline, respectively.

We revealed a frequent occurrence of multiple antimicrobial resistance and the presence of similar resistance patterns in *A. hydrophila, A. sobria* and *A. caviae* isolated from different food. It was mentioned that multiple resistance, particularly to ampicillin, cephalothin and chloramphenicol, is often seen in *Aeromonas* isolated from drinking water (Oliveira Scoaris et al., 2008). In the present study, we observed that about 15 % of the *Aeromonas* isolates were resistant to tetracycline (Tab. 3) similar to the findings of Oliveira Scoaris et al. (2008), who reported that 26 % of the *Aeromonas* strains were resistant to this antibiotic. These results are in contrast to the findings of Castro-Escarpulli et al. (2003) who have reported 44.1 % resistance to tetracycline. These microorganisms have been reported to be intrinsically resistant to ampicillin (Castro-Escarpulli et al., 2003; Oliveira Scoaris et al., 2008). When considered together, these data suggest that ampicillin and moxifloxacin should be avoided in the treatment of *Aeromonas* spp. infections. In this study, 60–65 % of the *Aeromonas* isolates were resistant to aztreonam and piperacillin (Tab. 3). This result is supported by Castro-Escarpulli et al. (2003) and Oliveira Scoaris et al. (2008). Our results showed that 65 % of the isolates were resistant to piperacillin, which was similar to the findings of Castro-Escarpulli et al. (2003) who have reported 19.4 % of piperacillin resistant strains.

In conclusion, the incidence of motile *Aeromonas* species (*A. hydrophila, A. sobria* and *A. caviae*) is high in food samples in Kırşehir (Turkey). It is reasonable to assume from the results obtained in this study that *Aeromonas* spp., whose growth yield increased substantially with proteolytic, lipolytic, nucleolytic and hemolytic activities at +37 °C and +25 °C, would have an extensive capacity for acting as pathogens in homeothermic hosts. So, the presence of *Aeromonas* with virulence attributes in water or food should always constitute a serious health hazard for humans and, therefore, its position regarding public health should be carefully revised. In addition, the results obtained in this study indicate that an increasing presence of multiple drug-resistant strains among *Aeromonas* species may become a potential danger for human health. To our knowledge this is the first report on the existence of virulence factors at different temperatures and the antimicrobial resistance of foodborne *Aeromonas* in Turkey.

TABLE 3: *Percentage of antimicrobial resistance of Aeromonas spp. isolated from food.*

Antibiotic concentration (µg/disc)		Percentage of resistant isolates: *A. hydrophila* (n=20)	*A. caviae* (n=12)	*A. sobria* (n=8)	Resistant isolates (%)
Imipenem	(10)	0.0	0.0	0.0	0.0
Ciprofloxacin	(5)	0.0	0.0	0.0	0.0
Tetracycline	(30)	15.0	16.6	12.5	15.0
Amoxicillin-clavulanic acid	(30)	70.0	75.0	87.5	75.0
Aztreonam	(30)	60.0	58.3	62.5	60.0
Piperacillin	(100)	65.0	83.3	37.5	65.0
Moxifloxacin	(5)	100	100	100	100
Ceptriaxone	(30)	0.0	0.0	0.0	0.0
Ceptazidime	(30)	25.0	33.3	37.5	30.0
Amikacin	(30)	0.0	0.0	0.0	0.0
Ampicillin	(10)	100	100	100	100

n: number of isolates

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