Research Journal of Biological Sciences 3 (8): 930-933, 2008
ISSN: 1815-8846

Investigating the Presence of *Staphylococcus aureus* and Coagulase Negative Staphylococci (CNS) in Some Leafy Green Vegetables

[1]M.E. Erkan, [1]A. Vural and [2]T. Özekinci
[1]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine,
University of Dicle, 21280 Diyarbakır, Turkey
[2]Department of Microbiology, Faculty of Medicine, University of Dicle,
21280 Diyarbakır, Turkey

Abstract: A total of 106 samples including parsley, lettuce, cress, watercress, mint and dill were analyzed for *Staphylococcus aureus* and Coagulase Negative Staphylococci (CNS). According to microbiological analyses, *Staphylococcus aureus* and the CNS were found in 8 (7.55%) and 48 (45.28%) samples, respectively. The CNS were identified as *S. epidermidis* 10 (9.43%), *S. arlettae* 9 (8.49%), *S. xylosus* 8 (7.55%), *S. sciuri* 6 (5.66%), *S. simulans* 4 (3.77%), *S. lentus* 3 (2.83%), *S. equorum* 3 (2.83%), *S. warneri* 3 (2.83%), *S. saprophyticus* 1 (0.94%) and *S. haemolyticus* 1 (0.94%).

Key words: *Staphylococcus aureus*, Coagulase Negative Staphylococci (CNS), vegetable, *S. epidermidis*

INTRODUCTION

More than 40 different species of *Staphylococcus* species have been identified until today (Bannerman, 2003; Kwok and Chow, 2003). *Coagulase positive S. aureus* is the most common infectious species for human beings and animals out of all these. While, Coagulase Negative Staphylococci (CNS) have been categorized as saprophytic and rare pathogenic until today, they are accepted to be an important pathogen for patients with weaken immune system and in infections related to medical equipments, in recent times. *S. epidermidis* was declared as a pathogen which is responsible for bacteriemy, infections of cerebrospinal fluid, peritoneal fluid, urinary system, prosthetic valve endocarditis and prosthetic joint infections. *S. haemolyticus* is the second most common CNS species for human beings and causes natural valve endocarditis, septicemia, infections of wounds, bones and joints and urinary system. *S. saprophyticus* was identified as an opportunistic pathogen which is responsible for urinary system infections in sexually active teenage women. Other CNS species are evaluated to be responsible for infections such as endocarditis, septicemia, pneumonia, infections of wounds and joints and osteomyelitis in human beings (Anonymous, 2005).

Coagulase negative Staphylococci (CNS) are principal component of skin and mucous membrane microflora of human beings (Minto *et al*., 1999). The information about the generation of CNS species in foods is limited. These species are rarely become the reason of food poisonings since they don't quickly multiply in foodstuff. However, some foodstuff can be contaminated by CNS species which were prepared by the patients with specific infections and human beings who are the common porters of these microorganisms (Bergdoll, 1995; Cunha *et al*., 2006).

The contamination of foodstuff with *Staphylococcus* species occurs in various stages from production to sales and distribution. Some CNS species such as *S. hyicus* (Hoover *et al*., 1983), *S. epidermidis* (Crass and Bergdoll, 1986), *S. xylosus* (Bautista *et al*., 1988), *S. warneri*, *S. saprophyticus*, *S. Ientus* (Valle *et al*., 1990) are also capable of enterotoxin production potential (Luca *et al*., 1997).

The purpose of this study is to demonstrate the presence of *S. aureus* and *coagulase negative Staphlylococci* in samples of parsley, lettuce, cress, watercress, mint and dill which are sold in Diyarbakır, in Turkey.

MATERIALS AND METHODS

Collection of the samples: One hunderd and six pieces of total samples of parsley (n: 22), lettuce (n: 20), cress (n: 16), watercress (n:18), mint (n:16) and dill (n:14) were taken into sterile sample bags, each to contain 250 g of sample, which were collected from various markets/

Corresponding Author: Aydin Vural, Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Dicle University, 21280, Diyarbakır, Turkey

greengrocers and bazaars in Diyarbakır. The samples were maintained in cold chain conditions (4°C), delivered to the laboratory immediately and analyzed.

Isolation and identification of *Staphylococcus* sp.: A piece of 11 g. were taken aseptically from each samples and homogenized in 99 mL. of buffered 0.1% peptone water. Samples from decimal dilutions were inoculated to Baird-Parker agar medium (Oxoid CM 275 B, SR 054). The typical colonies formed in medium were moved to Brain Hearth Broth medium (CM 225 B, Oxoid, Italy). In identification of isolated *Staphylococcus* sp., D Phoenix Automatic Microbiologic Identification System (BD Diagnostic Instrument Systems, Sparks, MD, USA) was also used additional to classical biochemical tests, in accordance with the instructions of the producer company (AOAC, 1995).

Phoenix ID/AST panel: BD Phoenix™ 100 automated microbiology identification system is a device which is designed for rapid identification of bacteria (ID) and antimicrobial sensivity tests (AST). Hundred identification and antimicrobial sensivity test can be run in this system. The ID section of the system contains a series of conventional, chromogenic and florogenic biochemical materials in order to identify the bacteria. Phoenix AST method is a broth based microdilutation test which is used for antimicrobial sensivity measurement. There are 45 micro-wells which contain dried chemical substrate and 2 fluorescence control micro-wells in ID section of the device. AST section contains 84 micro-wells with antimicrobial agents and a proliferation control micro-wells. The device was operated through placing pure cultures into it in accordance with the instructions of the Producer Company and run the fully-automated analyses procedure for evaluation.

RESULTS AND DISCUSSION

In this study, *S. aureus* in 8 green vegetable samples (7.55%) and *Coagulase* Negative *Staphylococci* (CNS) in 48 samples (45.28%) were isolated out of 106 samples total analyzed. The counts and rates of *S. aureus* and CNS in each sample were shown in Table 1 and 2. The highest *S. aureus* presence rate was in watercress as 22.22% and the highest CNS presence rate was in dill as 64.29%. *S. aureus* could not be isolated from any sample of lettuce, mint and dill. *S. haemolyticus* and *S. saprophyticus* were only isolated from 1 single samples of mint each while *S. hominis* and *S. capitis subsp. capitis* could not be isolated from any sample. The most common CNS isolated from the samples were *S. epidermis* (9.43%), *S. arlettae* (8.49%) and *S. xylosus* (7.55%).

During harvest process, vegetables and fruits can be contaminated with the pathogens from faecal materials, human hands, harvesting tools, containers used for carrying, transportation vehicles, wild and domestic animals, air and water (Beuchat, 1995).

S. aureus presence in the vegetables used for preparing salad was reported as 56.94% (41/72) by Viswanathan and Kaur (2001); 8.3% (3/36) by Satchell *et al.* (1990) and 5.1% (13/256) by Houang *et al.* (1991). Abdelnoor *et al.* (1983) have determined *Staphylococcus* count in lettuce and parsley samples as 14.3 and 7.7%, respectively. Ayçiçek *et al.* (2005) have reported *S. aureus* contamination in vegetable salads as 12.0% (9/75). *S. aureus* presence in leafy green vegetable samples involved in this study was 7.55% (8/106) and no *S. aureus* could be detected in lettuce, mint and dill samples. The differences between the results are probably due to analyzed samples, climate, season and hygienic conditions in storage and during sales.

S. aureus is a dangerous pathogen which is the most common reason of hospital infections (nosocomial infections) in human beings. The role of *Coagulase* negative *Staphylococci* in hospital infections was described over last two decades and *S. epidermidis* was declared as the most important infectious agent which is responsible for morbidity and mortality of these type of infectio infections (Ernesto *et al.*, 2002).

Udo *et al.* (1999) was isolated *S. aureus* and CNS species from hands of due to keeping the contaminated foodstuff under conditions suitable for toxin production. Besides the presences of *S. hominis*, *S. warneri* and *S. epidermidis* were determined as 23.6, 20.6 and 3.4%, respectively.

Rosec *et al.* (1997) was isolated 213 *S. aureus* and 51 *Coagulase* negative *Staphylococci* from 121 different foodstuff produced in France. The researchers reported that 30.5% of the *S. aureus* sp. were enterotoxin producing types but there were no enterotoxin producing bacterium among *Coagulase* Negative *Staphylococci*.

In a research in Spain conducted on nasal cavity of 300 food preparation workers, 27.6% *Coagulase* positive *Staphylococcus* (83/300) and 39.3 *Coagulase* negative *Staphylococcus* (118/300) were isolated. The CNS count in 64.4% of CNS porter food processors was reported as 10(3)-10(5) cob/swap (Francisco Polledo *et al.*, 1985).

It was reported that *Coagulase* Negative *Staphylococcus* sp. are present in normal microflora of skin and mucosal membranes of human beings, raw or cooked foodstuff can be contaminated by hand contacts and animals and CNS species can cause to Staphylococcus related food poisonings (Bergdoll, 1995; Udo *et al.*, 1999; Loir *et al.*, 2003; Cunha *et al.*, 2006). The research about the generation of CNS species in

Table 1: *S. aureus* presence rates and percentages in some leafy green vegetables

	Parsley (n: 22)	Lettuce (n: 20)	Lettuce (n: 20)	Watercress (n: 18)	Mint (n: 16)	Dill (n: 14)	Total (n: 106)
S. aureus	2 (9.09%)	ND*	2 (12.5%)	4 (22.22%)	ND	ND	8 (7.55%)

Table 2: The presence rates and percentages of *Coagulase* negative *Staphylococcus* sp. in some leafy green vegetables

	Parsley (n: 22)	Lettuce (n: 20)	Lettuce (n: 20)	Watercress (n: 18)	Mint (n: 16)	Dill (n: 14)	Total (n: 106)
S. arlettae	4 (18.18%)	2 (10.00%)	1 (6.25%)	ND*	ND	2 (14.29%)	9 (8.49%)
S. saprophyticus	ND	ND	ND	ND	1 (6.25%)	ND	1 (0.94%)
S. xylosus	3 (13.64%)	2 (10.00%)	ND	1 (5.56%)	ND	2 (14.29%)	8 (7.55%)
S. epidermis	3 (13.64%)	2 (10.00%)	1 (6.25%)	2 (11.11%)	ND	2 (14.29%)	10 (9.43%)
S. hominis	ND	ND	ND	ND	ND	ND	-
S. simulans	ND	ND	1 (6.25%)	1 (5.56%)	1 (6.25%)	1 (7.14%)	4 (3.77%)
S. haemolyticus	ND	ND	ND	ND	1 (6.25%)	ND	1 (0.94%)
S. lentus	ND	1 (5.00%)	ND	ND	2 (12.50%)	ND	3 (2.83%)
S. equorum	1 (4.55%)	ND	2 (12.50%)	ND	ND	ND	3 (2.83%)
S. sciuri	1 (4.55%)	1 (5.00%)	2 (12.50%)	ND	ND	2 (14.29%)	6 (5.66%)
S. warneri	ND	ND	1 (6.25%)	2 (11.11%)	ND	ND	3 (2.83%)
S. capitis subsp capitis	ND	ND	ND	ND	ND	ND	-
Total	12 (54.55%)	8 (40.00%)	8 (50.00%)	6 (33.33%)	5 (31.25%)	9 (64.29%)	48 (45.28%)

*ND: non detected

vegetable is limited. In this study, *S. aureus* in 8 leafy green vegetable samples (7.55%) and *Coagulase* Negative *Staphylococcci* (CNS) in 48 samples (45.28%) were isolated out of 106 samples total analyzed. By taking into consideration the results of this study, it can be suggested that those samples of green vegetables can be a serious risk factor for public health.

Food safety chain has to be implemented by application of Good Agricultural Practices (GAP), Good Hygiene Practices (GHP) and Hazard Analyses Critical Control Points (HACCP system), starting from irrigation of vegetable gardens and throughout all stages such as harvesting, transportation, storage and sales and processing of the products (Blumenthal *et al*., 2000; Anonymous, 2002). Keeping the green vegetables in cool conditions and separate sections from each other during sales and flushing out by clean water to remove raw contaminants can be recommended in order to decrease the bacterial counts on them. It is necessary to offer the vegetables for sale after they are washed, cleaned, disinfected and packaged by vacuum or under modified atmosphere, in order to eliminate the possible public health risk. Packaging under modified atmospheric conditions prolongs the shelf life of fresh vegetables and fruits and has growth inhibiting effects on pathogens (Jacxsens *et al*., 1999).

REFERENCES

Abdelnoor, A.M., R. Batshoun and B.M. Roumani, 1983. The bacterial flora of fruits and vegetables in Lebanon and the effect of washing on the bacterial content. Zbl. Bakt. Hyg., I. 9 Abt. Orig. B., 177: 342-349.

Anonymous, 2002. Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw. European Commission Health and Consumer Protection Directorate-General SCF/CS/FMH/SURF/ Final. Brussels.

Anonymous, 2005. The Medical Significance and Identification of Coagulase Negative Staphylococci. Microgen Bioproducts Newsletter, 22: 1-4.

AOAC, 1995. Bacteriological Analytical Manual. 8th Edn. Association of Official Analytical Chemist (AOAC) International. Gaithersburg, USA.

Ayçiçek, H., S. Cakıroglu and T.H. Stevenson, 2005. Incidence of *Staphylococcus aureus* in ready-to-eat meals from military cafeterias in Ankara, Turkey. Food Control, 16: 531-534.

Bannerman, T.L., 2003. *Staphylococcus*, Micrococcus and Other Catalase-Positive Cocci that Grow Aerobically. In: Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Yolken (Eds.). Manual of Clinical Microbiology, Washington: Am. Soc. Microbiol., pp: 384-22, 404.

Bautista, L., P. Gaya, M. Medina and M.A. Nunez, 1988. Quantitative study of enteroxin production by sheep milk Staphylococci. Applied Environ. Microbial., 54: 566-569.

Bergdoll, M.S., 1995. Importance of Staphylococci that produce 1 nanogram quantities of enterotoxin. Zbl Bakt., 282: 1-6.

Beuchat, L.R., 1995. Pathogenic microorganisms assosiated with fresh produce. J. Food Prot., 59: 204 -216.

Blumenthal, U.J., D.D. Mara, A. Peasey, G. Ruiz-Palacios and R. Stott, 2000. Guidelines for the microbiological quality of treated wastewater used in agriculture: Recommendations for revising WHO guidelines. Bull. World Health Org., 78: 1104-1116.

Crass, B.A. and M.S. Bergdoll, 1986. Involvement of Coagulase-negative staphyiococci in toxic shock syndrome. J. Clin. Microbial., 23: 43-45.

Cunha, M.L.R.S., E. Peresi, R.A.O. Calsolari and J.P.A. Junior, 2006. Detection of enterotoxins genes in Coagulase-Negative Staphylococci isolated from foods. Brazilian J. Microbiol., 37: 70-74.

Ernesto Calderón-Jaimes, M.C.M.S.P., Luz E Espinosa de los Monteros, C. Dra en and Renata M.C. Avila-Beltrán, 2002. Epidemiology of drug resistance: The case of Staphylococcus aureus and Coagulase-negative Staphylococci infections. Salud Pública De México, 44: 108-112.

Francisco Polledo, J.J., M.L. Garcia, B. Moreno and I. Menes, 1985. Importance of food handlers as a source of enterotoxigenic Staphylococci. Zentralbl. Bakteriol. Mikrobiol. Hyg. (B), 181: 364-373.

Hoover, D.G., S.R. Tatini and J.B. Maltais, 1983. Characterization of Staphylococci. Applied Environ. Microbial., 46: 649-660.

Houang, E., P. Bodnaruk and Z. Ahmet, 1991. Hospital green salads and the effects of washing them. J. Hosp. Inf., 17: 125-131.

Jacxsens, L., F. Devlieghre, P. Falcato and J. Debevere, 1999. 1 Behavior of *Listeria monocytogenes* and *Aeromonas* sp. on fresh-cut produce packaged under equilibriummodified atmosphere. J. Food Protec., 62: 1128-1135.

Kwok, A.Y.C. and A.W. Chow, 2003. Phylogenetic study of *Staphylococcus* and *Micrococcus* sp. based on partial hsp60 gene sequences. Int. J. Syst. Evol. Microbiol ., 53: 87-92.

Loir, L.L., F. Baron and M. Gautier, 2003. *Staphylococcus aureus* and food poisoning. Genet. Mol. Res., 2: 63-76.

Luca, G.D., F. Zanetti and S. Stampi, 1997. *Staphylococcus aureus* in dairy products in the Bologna area. Int. J. Food Microbiol., 35: 267-270.

Minto, E.C.M., C. Barelli, R. Martinez and A.L.C. Darini, 1999. Identification and medical importance of Coagulase-negative Staphylococci sp. Sao Paulo Med. J. Rev. Paul. Med., 117: 175-178.

Rosec, J.P., J.P. Guiraud, C. Dalet and N. Richard, 1997. Enterotoxin production by Staphylococci isolated from foods in France. Int. J. Food Microbiol., 35: 213-221.

Satchell, F.B., P. Stephenson, W.H. Andrews, L. Estela and G. Allen, 1990. The survival of *Shigella sonnei* in shredded cabbage. J. Food Prot., 624 (53): 558-562.

Udo, E.E., M.A. Al-Bustan, L.E. Jacob and T.D. Chugh, 1999. Enterotoxin production by Coagulase-negative Staphylococci in restaurant workers from Kuwait City may be a potential cause of food poisoning. J. Med. Microbiol., 48: 819-823.

Valle, J., E. Gomez-Lucia, S. Piriz, J. Goyachc, J.A. Orden and S. Vadillo, 1990. Enterotoxin production by Staphylococci Isolated from Healthy Goats. Applied Environ. Microbiol., 56: 1323-1326.

Viswanathan, P. and R. Kaur, 2001. Prevalence and growth of pathogens 1 on salad vegetables, 2 fruits and sprouts. Int. J. Hyg. Environ. Health, 203: 205-213.