Bacteria Associated with the Spoilage of Salad, their resistotyping and Potential Public Health Implications

[1]Adegoke Anthony A., [2]Divine-Anthony O.

[1,2]Department of Microbiology, University of Uyo, Uyo, Akwa Ibom State, Nigeria

Abstract— *A study of bacteria associated with spoilage of various types of salads purchased from 3 different restaurants in Uyo metropolis was carried out between the months August and October, 2008 using standard microbiological procedures. The mean microbial load in vegetable salad was (3.5±0.25) x 10^7cfu/g with the sample from food affairs having the highest load (3.7 x 10^7cfu/g). The mean microbial load in garlic salad was (3.2±0.25) x 10^7cfu/g also with the sample from food affairs having the highest load (3.6 x 10^7cfu/g). However, fruit salad samples from Mr. Biggs had the highest microbial load (3.9 x 10^7cfu/g) out of the mean value of (3.6±0.25) x 10^7 cfu/g. Analysis of the frequency of occurrence of the isolates revealed that Staphylococcus aureus (33%), Micrococcus spp. (20%), Bacillus spp. (20%), Proteus spp. (13%), Pseudomonas spp. (13%) and Escherichia coli (7%) were the most encountered organisms in both spoiling and fresh (control) salad. The resistotyping of 20 % of tested bacteria were resistant to 5 antibiotics. The presence of Escherichia coli and some potential pathogenic microorganisms in all the samples collected are indications of faecal contamination and unhygienic method of food handling. This suggests that salad should be properly managed during and after preparations to avoid outbreak of food borne diseases. Also the food handlers should be trained and educated on the importance of sanitary condition during food production.*

Keywords— Bacteria, salad, spoilage, Staphylococcus species, Escherichia coli, resistotyping.

I. MATERIALS AND METHODS

A. Sources and Collection of Salad

Samples used in this work were purchased from 3 different fast food restaurants in Uyo metropolis. They were aseptically packaged prevent contaminations during transportation to laboratory. They were analyzed immediately to determine the bacterial load and diversities associated with fresh salad. The left over were repackaged and allowed to stand without refrigeration until the signs of spoilage were observed. Samples from the spoilt salad were re-analyzed as done to the fresh fruit (control). The rationale for determining the bacterial load and diversities in the fresh salad is to source track the bacterial isolates that would be determined later while spoiling.

B. Media, Reagents and Antibiotics

Media used which include Nutrient Agar (Oxoid, Cambridge, UK) macConkey Agar (Oxoid, Cambridge, UK), Eosin Methylene Blue Agar (Oxoid, Cambridge, UK) and reagents were purchased from Globask Scientific, Idumota, Lagos. The single antibiotic disks were purchased from Oxoid representative in Ogba, Ikeja, Lagos, Nigeria.

C. Determinations of Bacterial Load and Bacterial Diversities

Established 10 fold serial dilution methods of Collins and Lyne (1979) was employed to determine the total heterotrophic bacterial count and coliform count using nutrient agar and Eosin Methylene Blue Agar (Oxoid, Cambridge, UK) respectively. Determination of bacterial diversities in both fresh (control) and the spoiling salad was carried out using conventional methods described by Cheesebrough (2006) and Kit systems.

D. Resistotyping of the identified Bacterial Species:

Disc diffusion techniques as described by Cheesebrough (2006) was used for this test. The selected antibiotics were nalidixic acid, kanamycin, amoxicillin-clavulanic acid, oxytetracycline, cefotaxime and enrofloxacin.

II. RESULTS

Evidence of spoilage observed in the unrefrigerated salad several hours after purchase include change in colour, taste and obvious sign of primary decay in the sliced fruits.

A. Microbial Counts of the Salad Samples

The mean microbial load in vegetable salad was (3.5±0.25) x 10^7cfu/g with the sample from Fast Food FA having the highest load (3.7 x 10^7cfu/g). The mean microbial load in garlic salad was (3.2±0.25) x 10^7cfu/g also with the sample from Fast Food FA having the highest load (3.6 x 10^7cfu/g). However, fruit salad samples from Fast Food MB had the highest microbial load (3.9 x 10^7cfu/g) out of the mean value of (3.6±0.25) x 10^7 cfu/g. The bacterial load in the freshly prepared salad was lower than the load observed in the spoiling salad. The least load was observed in garlic salad (Table 1and 2)

B. Bacterial Diversities common in all the Salad during Spoilage and their Frequency of Occurrence

The bacterial S*taphylococcus aureus*, *Proteus vulgaris*, *Bacillus* spp., *Psedomonas* spp., *Micrococcus* spp., *Escherichia coli.* All the bacterial diversities were detected in both the freshly prepared and spoiling salad. The analysis of the frequency of occurrence of the isolates revealed that *Staphylococcus aureus* has the highest frequency of occurrence while the *Micrococcus* spp., *Bacillus* spp., *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli* were the most encountered organisms (Table 3)

TABLE 1
BACTERIAL COUNTS FROM THE UNREFRIGERATED SALAD UNDERGOING SPOILAGE AND CONTROL (FRESHLY PREPARED)

Fast Food Outfits	**Sample type 1 Veg. (cfug^{-1}) x 10^7**	**Sample type 1 Control Veg Salad (cfug^{-1}) x 10^3**	**Sample type 2 Garlic Salad (cfug^{-1}) x 10^7**	**Sample type 2 Control Garlic Salad (cfug^{-1}) x 10^3**	**Sample type 3 Fruit Salad (cfug^{-1}) x 10^7**	**Sample type 3 Control Fruit Salad (cfug^{-1}) x 10^3**
MB	3.5	1.7	3.3	1.3	3.9	1.4
FA	3.7	1.1	3.6	1.2	3.6	1.5
OT	3.4	1.4	2.8	0.8	3.4	1.3
MEAN	3.5	1.4	3.2	1.1	3.6	1.4

TABLE 2
COLIFORM COUNTS FROM THE UNREFRIGERATED SALAD UNDERGOING SPOILAGE AND CONTROL (FRESHLY PREPARED)

Fast Food Outlet	**Sample 1 Vegetable Salad ($cfug^{-1}$)** x 10^5	**Sample 1 Control Veg Salad ($cfug^{-1}$)** x 10	**Sample 2 Fruit Salad ($cfug^{-1}$)** **x** 10^5	**Sample 2 Control F Salad ($cfug^{-1}$)** **x** 10	**Sample 3 Garlic Salad ($cfug^{-1}$)** **x** 10^3	**Sample 3 Control Garlic Salad ($cfug^{-1}$)**
MB	1.8	0.7	2.0	1.0	2.3	6
FA	2.7	1.1	2.3	.	2.5	7
OT	2.1	1.2	1.5	1.5	1.9	6
ẍ	2.2	1.0	1.9	1.9	2.2	8

TABLE 3
FREQUENCY OF OCCURRENCE OF BACTERIA AND COLIFORM IN THE THREE SALAD SAMPLES.

S/N	**Type of Organisms**	**Frequency of Occurrence (%)**
1	*Staphylococcus* spp	33
2	*Proteus* spp	20
3	*Bacillus* spp	20
4	*Pseudomonas* spp	13
5	*Escherichia coli*	7
6	*Micrococcus* spp	7
		100

TABLE 4
RESISTOTYPING OF THE BACTERIAL ISOLATES

NA	**C**	**AUG**	**OT**	**CTX**	**ENF**
3S 3I 5R	8S 1I 2R	5S 6R	8S 3R	2S 1R 8R	100S
27.7S	72.8S	45.5S	72.8S	18.2S	
27.7I	9I	55.5R	27.2R	9I	
45.6R	18.2R			72.8R	
20% were Resistant to 5 Antibiotics					

Key: NA= nalidixic acid, C= kanamycin, AUG= amoxicillin-clavulanic acid, OT=oxytetracycline, CTX= cefotaxime, ENF= enrofloxacin

III. DISCUSSION

The salad analysed in this study is an important food supplement because it is made from various vegetables and fruits which includes cabbage, white onion, carrot, cucumber, green peas, lettuce, spinach and cream salad. The consumption of spoilt or contaminated salad will pose a greater danger to health because of the uncooked nature by which the salad is usually served. Since the salad samples analysed in this study indicate the presence of some potential pathogenic microorganisms, the knowledge of the bacterial flora of salad is necessary in order to effectively safeguard against the consumption of contaminated salad.

Analysis of the frequency of occurrence of the isolates in this study revealed that *Staphylococcus aureus, Micrococcus spp, Bacillus spp, Proteus spp, Pseudomonas spp, Escherichia coli* were the most encountered organisms. Deng *et al.,* (1998) emphasized the ability of this bacterium to survive in dry foods, with a wide range of aw and pH values, particularly at refrigeration temperatures. Abdul Raouf *et al.,* (1993) have reported that populations of *E. coli* O157: H7 (10^5 cfu/g) inoculated in ground, roasted beef salads containing up to 40 % mayonnaise and held at 5 ºC showed no changes even after 72 hr of incubation. *Staphylococcus aureus* is distributed in the environment and their primary habitant is the anterior nares and skin of man and animals (Adegoke and Komolafe, 2009). Bacillus spp can grow in food and produce enterotoxins that cause food poisoning. Both bacterial species are widely distributed in the soil, they often contaminated vegetable and fruits. Most of these organisms have been implicated in septicaemia (Komolafe and Adegoke, 2008).

Other organisms found include proteus sp and micrococcus sp. The presence of *Escherichia coli* in food, water and fruits is an indication of faecal contamination resulting from unhygienic method of food handling. The occurrence of pseudomonas sp in salad also can constitute a public health hazard, especially because of their high resistance to antibiotics. This types of multidrug resistance of 20 % to 5 last line antibiotics make the isolated bacteria of high epidemiological importance (Adegoke and Komolafe, 2009).

IV. CONCLUSION AND RECOMMENDATION

This study has shown that salad although very delicious and nutritive do harbour microorganisms including pathogenic microorganism. The activities of microorganisms have been reported as the main cause of salad spoilage. The source of the microorganisms isolated in this study is suspected to come from the poor handled conditions and their proliferation enhanced.

Based on the result of this study, there is need that ready to eat food should be serve immediately preparation because result of this analysis has shown that some microorganisms can grow and multiply in the food item even under refrigeration. Producers of salad should make sure that the condiments used for the production of salad are of recommended microbiological standards. It is also important that food handlers are trained and educated on the importance of sanitary condition during food production.

REFERENCES

[1] Abdul-Raouf, U., Beuchat, L. and Ammar, M. (1993). Survival and growth of Escherichia coli O157: H7 in ground, roasted beef as affected by pH, acidulant and temperataure. Applied Environmental Microbiology 59: 2364-2368.

[2] Adegoke, A. A., and Komolafe, A. O. (2009) Multi-drug resistant Staphylococcus aureus in clinical cases in Ile-Ife, Southwest Nigeria., International Journal of Medicine and Medical Science, 1(3): 068 – 072.

[3] Brown, G.K. (2002). Development in Technology of Storage and Handling of Fresh Fruits and Vegetables. Critical Review Food Technology. Pp 15-54.

[4] CDC (Centers for Disease Control and Prevention), (1996). Outbreak of Escherichia coli 0157:H7 Infections Associated with Drinking of Unpasteurized Commercial Apple Juice British Columbia, California, Colorado, Washington, October, 1996. Morbid, Mortality Weekly Report 45, 975.

[5] Cook, K.A., Dobbs, T.E., Hlady, W.G., Wells, J.G., Barrett, T.J., Puhr, N.D., Lancette, G.A., Bodager, D.W., Toth, B.L., Genese, C.A., Highsmith, A.K., Pilot, K.E., Finelli, L., Swerdlow, D.L. (1998). Outbreak of Salmonella serotype Hartford Infection Associated with Unpasteurized Orange Juice. JAMA 280(17), 1504-1509.

[6] Deng, Y., J. Ryu and L. Beuchat. (1998). Influence of temperature and pH on survival of Escherichia coli O157: H7 in dry foods and growth in reconstituted infant rice cereal. International Journal of Food Microbiology 45: 173-184.

[7] Gaylor, G.E., MacCready, J.P., Reardon, J.P., McKernan, B.F. (1995). An Outbreak of Salmonellosis Traced to Waterlemon. Public Health Report 70, 311-313.

[8] Grawmlich, T.R., Marcy, J.E., Adams, J.P. (1986). Aseptically Packaged Orange Juice and Concentrate: A Review of the Influence of Processing and Packaging Conditions on Quality. Journal Agriculture and Food Chemistry 34, 402-405.

[9] Hatcher Jr., W.S., Parish, M.E., Weih, J.L., Splittstoesser, D.F., Woodward, B.B. (2000). Fruit Beverages. In: Downes, F.P., Ito, K. (Eds), Methods for the Microbiological Examination of Foods. American Public Health Association, Washington DC. Pp 565-568.

[10] Itah, A.Y. and Opara, A.A. (1997). Enterotoxin Production by Staphylococcus aureus Contaminating Canned Foods in Calabar, Nigeria. Journal of Science Engineering and Technology. 740-750.

[11] Komolafe, A.O. and Adegoke, A.A. (2008) Incidence of Bacterial Septicaemia in Ile-Ife Metropolis, Nigeria. Malaysian Journal of Microbiology, 4, 51-61

[12] Krause, G., Terzagian, R., Hammond, R. (2001). Outbreak of Salmonella Serotype Anatum Infection Associated with unpasteurized orange juice. South African Medical Journals 94(12), 1168-1172.

[13] Paris, M.E., 1998. Coliforma, Escherichia coli and Salmonella Serovars Associated with a Citrus Processing Facility in a Salmonellosis Outbreak. Journal of Food Protection 61(3), 280-284.

[14] Varma, S.K., Varma, R.A. (1987). Aflatoxin B1 Production in Orange (Citrus reticulate) juice by Isolates of Aspergillus flavus Link. Mycopathogenic 97, 101-104.