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Short Communication

The microbial quality of pre-packed mixed vegetable salad in some retail outlets in Lagos, Nigeria

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Pre-packed mixed vegetable salad and salad ingredients- carrots, cucumber, cabbage, and lettuce were analyzed for their microbial quality. The salads were obtained from fast food outlets (well packaged at 4 °C) and open markets (exposed at 35°C) within Lagos metropolis. The analysis was both qualitative and quantitative. Microorganisms isolated from salad samples from fast food outlets include *Aspergillus fumigatus, Trichoderma* spp, *Staphylococcus aureus* and *Proteus mirabilis*, while those isolated from open market samples include *Mucor* spp, *A.* fumigatus, *Aspergillus niger, Trichoderma* spp, *Neurospora* crassa, *Proteus vulgaris*, *S. aureus*, *Citrobacter freundii, Proteus mirabilis,* and *Corynebacterium* spp. Those from salad ingredients include *Mucor* spp, *A. fumigatus*, *Trichoderma* spp, *N. crassa*, *Rhizopus* spp, *A. niger*, *P. vulgaris*, *P. mirabilis*, *S. aureus, Pseudomonas aeruginosa* and *C. fruendii.* The total viable count was highest in salad samples from open markets (5.9×10^6 cfu/g) and lowest in salad samples from fast food outlets (2.6×10^4 cfu/g). The total viable counts obtained from the salad ingredients were generally lower than those obtained from salads. Among the salad ingredients the highest count was however obtained from carrot (3.0×10^2 cfu/g) and lowest count from cucumber (1.3×10^2 cfu/g). Gentamicin, chloramphenicol, cotrimoxazoleoflaxacin were most effective against the bacterial isolates yielding greater zones of inhibition. The storage temperature and the dirty nature of the open markets must have been responsible for the occurrence of more microorganisms in salad samples from open markets than those from fast food outlets. The need for safe salad can not be overemphasized.

Key words: Vegetable salad, salad ingredients, microorganisms, fast food outlets, open markets.

INTRODUCTION

Salad is a term broadly applied to many food preparations that have a mixture of chopped or sliced ingredients which may be mainly fruits or vegetables. The inner tissues of healthy plants and animals are free of microorganisms. However, the surfaces of raw vegetables and meats are contaminated with a variety of microorganisms and this depends on the microbial population of the environment from which the food was taken, the condition of the raw product, the method of handling, the time and conditions of storage (Pelczar et al., 2006).

Bacteria involved in spoilage of vegetables are usually pectinolytic species of the Gram negative genera of *Erwinia*, *Pseudomonas*, *Clostridium*, and *Xanthomonas* and the non-sporing Gram positive organisms like *Corynebacterium* (Adams and Moss, 1999). Salads containing raw vegetables have been identified as vehicles of traveller's diarrhea, an illness sometimes experienced by visitors to developing countries (Beuchat and Larry, 1996). The microbial flora of partially processed vegetables as found in pre-packed mixed vegetable salads should be of great concern since both food spoilage and safety are involved. The presence or absence as well as the fate of both human pathogens and plant tissue spoilage organisms are important. The objective of this study therefore is to isolate and identify the microorganisms present in pre-packed mixed vegetable salads from different retail outlets in Lagos, Nigeria, as this will help in establishing the effect of environment where the food was taken, type of handlingand type of storage condition on the microbial quality of the product.

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Table 1. Total viable count of salad samples and salad ingredients.

Salad samples			
Locations	**Total viable count (cfu/g)**	**Salad ingredients**	**Total viable count (× 10^2cfu/g)**
Yaba market	5.9×10^6	cucumber	1.3
Bariga market	5.7×10^6	carrot	3.0
Fast food outlet 1(F_1)	3.3×10^3	lettuce	2.0
Fast food outlet 2(F_2)	2.6×10^4	cabbage	2.1

Table 2. Occurrence of microorganisms in salad and salad ingredients.

Isolates	**Fast food outlets**		**Open markets**		**Salad ingredients**			
	F_1	**F_2**	**Yaba**	**Bariga**	**Cucumber**	**Carrot**	**Lettuce**	**Cabbage**
Mucor spp	-	-	-	+	+	-	-	+
A. fumigatus	+	-	+	+	-	+	+	+
Trichoderma spp	+	-	+	+	-	-	+	-
N. crassa	-	-	+	+	+	+	-	-
Rhizopus spp	-	-	-	-	+	+	+	-
A. niger	-	-	+	+	+	+	-	-
P. vulgaris	-	-	+	+	+	-	-	+
S. aureus	+	+	-	+	+	-	+	+
C. freundii	-	-	-	+	-	+	+	-
P. mirabilis	+	-	+	+	-	-	-	+
P. aeruginosa	-	-	-	-	+	-	-	+
Corynebacterium spp	-	-	+	+	-	-	-	-

MATERIALS AND METHODS

Collection of samples

Pre-packed mixed vegetable salads were obtained from fast food outlets stored at 4°C in refrigerators located in clean and enclosed buildings (F_1 and F_2) and dirty open markets (Yaba and Bariga) where wares and food items are displayed in the open at temperature of 35°C. Salad ingredients: carrots, lettuce, cabbage, and cucumber were also obtained from the market. The fast food outlets are in better hygienic condition than the open markets which are very dirty.

Isolation of microorganisms

From each salad sample, 25 g was aseptically weighed and macerated and 225 mls of sterile distilled water was added. Serial dilution was carried out using sterile distilled water as diluents. From each dilution 1 ml was plated using the pour plate method. The culture media used were nutrient agar, MacConkey agar and potato dextrose agar. Plating was done in duplicates. Nutrient agar plates and MacConkey agar plates were incubated at 37°C for 24-48 h while the potato dextrose agar plates were incubated at 25°C for 48-72 h. For the salad ingredients the procedure explained above was done for each of them.

After incubation, colonies that developed on the plates were counted to obtain total viable count. Pure cultures of the isolates were obtained by subsequent sub-culturing on fresh agar plates.

Identification of microbial isolates

This was done based on cultural, morphological and biochemical characteristics of the isolates using standard methods (Buchanan and Gibbons, 1974; Talbot, 1971).

Antibacterial susceptibility testing

Broth culture of each bacterial isolate was swabbed on Mueller Hinton agar plates with sterile swab sticks. Commercial antibiotic discs were placed on the inoculated plates, which were subsequently incubated at 37°C for 24 h. After incubation, the plates were examined for zones of inhibition. The degree of sensitivity was expressed as a measure of the diameter of zones of inhibition of growth in millimeters.

RESULTS

The total bacterial count was higher in salad samples from the open markets than those from fast food outlets. The salad ingredients analyzed had the least bacterial count (Table 1).

The microorganisms isolated from salads and salad ingredients include Citrobacter fruendii, *Proteus vulgaris*, *Proteus mirabilis*, *Staphylococcus aureus*, *Mucor* spp, *Aspergillus fumigatus*, *Trichoderma*, *Neurospora crassa* and *Aspergillus niger*. *Corynebacterium* spp was isolated from only salad samples collected from open markets. *Pseudomonas aeruginosa* and *Rhizopus* spp were isolated from only salad ingredients. More microorganisms occurred in salads obtained from the open markets than in salads obtained from fast food outlets (Table 2).

Most bacterial isolates were more sensitive to gentami-

Table 3. Antibiotic sensitivity of isolated Gram positive bacteria.

Antibiotics	Zones of inhibition (mm)	
	Staphylococcus aureus	***Corynebacterium* sp**
Amoxycillin	25	18
Erythromycin	25	19
Tetracycline	27	26
Cloxacillin	16	14
Chloramphenicol	30	30
Cotrimoxazole	30	17
Augmentin	27	17
Gentamycin	29	24

Table 4. Antibiotic sensitivity of isolated Gram negative bacteria.

Antibiotics	Zones of inhibition (mm)			
	C. freundii	***P. vulgaris***	***P. mirabilis***	***P. aeruginosa***
Gentamicin	26	27	24	21
Nalidixicin acid	19	-	28	-
Oflaxacin	29	30	29	29
Augmentin	15	14	-	-
Tetracycline	28	16	16	-
Amoxycillin	-	17	-	-
Cotrimoxazole	17	15	-	-
Nitrofurantoin	-	16	18	-

-: No sensitivity.

cin, chloramphenicol, contrimoxazole and oflaxacin (Tables 3 and 4).

DISCUSSION

The higher bacterial counts obtained from salad samples as compared to salad ingredients with lower bacterial count may be due to handling and non hygienic practices during salad preparation which may have led to introduction of additional microorganisms into the salad. Exposure of salad to contaminants during storage and retail may have also contributed. On the other hand, growth of the initial contaminants during storage of salad may have contributed to the higher bacterial counts, especially with salad in the open markets which were stored at ambient temperatures. Salad samples from the fast food outlets yielded lower numbers and types of microbial isolates than the market samples. This could be as a result of the relatively clean environment in the fast food outlets when compared to the dirty open markets. The storage temperature of 4^{o}C in the fast food outlets compared to 35^{o}C in open markets may also be responsible. From results obtained from this work, different types of bacteria and molds were isolated from vegetable salads. Vegetables, fruits, pulses, oil seeds and their products carry microorganisms such as fungi, actinomycetes and bacteria (Majumder, 1994). *Pseudomonas* spp is a prominent inhabitant of soil and water. The organism is responsible for diseases of vegetables like angular leaf spot of cucumber. *S. aureus* is of health significance as it can cause food poisoning. However, the need for good hygienic practices, proper handling, storage and retail of salads in clean environment and at refrigeration temperature can not be over emphasized to ensure good quality and safe salads.

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