

Potential Human Pathogens (Bacteria) and their Antibigram in Ready-to-eat Salads sold in Calabar, South-South, Nigeria

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Citation

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Abstract

A total of 150 ready-to-eat salad samples were collected from various sources in Calabar and analyzed bacteriologically using standard cultural methods. The samples included 50 from fast food centers, 50 from wedding reception parties and 50 from birthday reception parties all within Calabar metropolis between October 2006 and January 2007. All the bacterial isolates were characterized using the API system and subjected to antimicrobial susceptibility test using the Agar diffusion method. The probable human pathogens isolated were *Staphylococcus aureus* 35 (41.18%), *Escherichia coli* 21 (24.71%), *Klebsiella aerogenes* 11 (12.94%), *Salmonella Enterica* var *Typhimurium* 3 (3.53%), and *Bacillus* species 15 (17.65%). Rates of contamination in relation to the various sources were: samples from fast food centers 40.0%, birthday party samples 52.0% and 78.0% for samples from wedding reception parties. Antimicrobial susceptibilities ranged from 63.64% to 84.85%. These findings show that ready to eat salads are commonly contaminated with potential human pathogens that carry multiple antimicrobial resistant genes. High standards of personal hygiene, pre-processing disinfection and proper storage temperatures should always be observed during preparation and distribution of fresh vegetable and fruit salads.

INTRODUCTION

Salad is a mixture of fresh vegetables and cream milk that provides a rich source of vitamins, minerals and dietary fiber of low fat and calories to the consumer. However, questions have been raised about the safety and microbiological quality of these food produce [1]. Several outbreaks of human gastro-enteritis have been linked to the consumption of contaminated fresh vegetable salad [23].

In developing countries, there are serious concerns about the sanitation of ready to eat salads, particularly as potable water is seldom available at preparation venues and the fast food stands. Washing of dishes and utensils is often with a single change of water in the same basin all day long [4]. Health risks are associated with initial contamination and subsequent contaminations by the workers during handling while microbial proliferation occur during display. Salads are usually consumed without any pre-heat treatment and therefore the possibility of food poisoning exists [5].

The increasing availability of prepared vegetable salads reflects consumers demand for fresh, healthy, convenient and additive free foods that should be safe and nutritious.

The survival of food borne pathogens is slightly enhanced once the protective epidermal barrier has been broken either by physical damage or by degradation by plant pathogens [6]. These punctures can also promote the multiplication of pathogens especially at non-refrigerated temperatures [7]. During salad preparation, raw vegetables are injured through peeling, slicing or shredding [8]. These operations can transfer pathogenic microorganisms if present on the surface of fresh fruits and vegetables into the product. Most pathogens in salad do not cause product spoilage, even at relatively high populations. In the absence of spoilage signs, salads are consumed because they are perceived as safe. Several studies have associated outbreak of food poisoning with consumption of raw vegetable salads [9,10,11].

In recent years, salad has become a very popular component of menu served at birthday and wedding parties; they are also sold in fast food centers in most major cities in Nigeria. Media reports of unverified rampant cases of gastroenteritis following consumption of meals served with fresh vegetable salads have become serious public health concern. This study was therefore set up to evaluate the microbial quality of ready to eat salads commonly consumed in Calabar,

South-South Nigeria The study will also address the profile of multiple drug resistance among isolates from ready to eat salads.

MATERIALS AND METHODS

Study Location: Calabar, is the capital city of Cross River State and one of the fastest growing metropolis in Nigeria. In the last 9 years, the metropolis has been transformed from a mere sub-urban community to become Nigeria’s tourist destination. The hospitality industries have also experienced phenomenal growth with more sophisticated appetite. With Tinapa an international resort and shopping empire coming on stream well over ten million tourists are expected to visit Calabar every year. . The population of Calabar is approximately one million; the people are principally civil servants, traders, farmers and fishermen.

Sample Collection: Sampling for this study lasted between January 2007 and October 2007. A total of 150 salad samples were collected with 50 samples from fast food centers, birthday reception parties and wedding reception parties respectively. Only one sample each was collected from each location.

The samples were transported to the microbiology laboratory for processing on the same day of collection. Where delays were inevitable, the samples were stored at 4^{OC} overnight and allowed to reach room temperature before they were processed.

One gram of thoroughly mixed salad sample was aseptically added to 18ml of sterile Thioglycollate broth (USP) and incubated overnight at 37^{OC}. After 24 h. incubation, the thioglycollate broth was sub-cultured onto blood agar, chocolate agar, MacConkey agar and thiosulphate citrate bile salt agar (TCBS) plates All the plates were incubated aerobically except the blood agar plates which were incubated anaerobically with gas pack for 18-24 hr at 37^{OC}. The isolates were fully characterized using the API system.

ANTIMICROBIAL SUSCEPTIBILITY TEST

All the isolates that were potential human pathogens were subjected to antimicrobial susceptibility test using the agar diffusion method on Mueller Hinton agar [12]. The antimicrobial agents used and the disk concentrations were Benzyl penicillin 1.5 units, Ampicillin 25mcg, Tetracycline 10mcg/ 25mcg for gram positive and gram negative organisms respectively, Chloramphenicol 25mcg,Gentamicin 10mcg, Ciprofloxacin 10mcg,Cloxacillin

10mcg Cotrimoxazole 25mcg, Ceftazidime 10mcg, and Augmentin 30mcg. Laboratory maintained susceptible strains were used as controls in the susceptibility tests.

RESULTS

In this study a total of 150 salad samples were examined for bacterial contamination. In all, 85(56.67%) of the salad samples were contaminated with various bacterial species belonging to 5 genera, 3(60.0%) being gram negatives while 2(40.0%) were gram positives (see table1) below. A total of 35(41.18%) of the salad samples studied were contaminated with Staphylococcus aureus .The isolation rates for the other bacteria generally considered as potential human pathogens were: E. coli 21(24.71%), Kleb. aerogenes 11(12.84%), Salmonella Enterica var Typhimurium 3(3.53%) and Bacillus spp. 15(17.65%). Salad samples obtained at wedding reception parties had the highest contamination rate of 78.0 percent followed by birthday party samples 52.0 percent. Only 40.0percent of samples from fast food centers were contaminated with potential human pathogens. There is a strong significance difference in the contamination rates among wedding party samples and the samples from fast food centers (p<0.001).

Figure 1

Table 1: Distribution of bacterial species in ready to eat salad samples from various sources in Calabar.

Bacterial Species	No.(%) of isolates from various sources			Total no of samples no.=150
	Fast food Centers No.=50	wedding parties no.=50	birthday parties no.=50	
S. aureus	6(30.0)	17(43.59)	12(46.15)	35(41.18%)
E. coli	5(25.0)	9(23.08)	7(26.92)	21(24.71%)
Kleb.aerogenes	2(10.0)	6(15.38)	3(11.54)	11(12.84%)
Salm. Spp.	0(0.0)	2(5.13)	1(3.85)	3(3.53%)
Bacillus spp.	7(35.0)	5(12.82)	3(11.54)	15(17.65%)
Total	20(40.0%)	39(78.0%)	26(52.0%)	85(56.67%)

In table 2 below is presented the Antimicrobial resistance profiles of the potential human pathogens isolated from fresh vegetable salads in Calabar, South-south Nigeria. The isolates showed variable resistance profiles ranging from 25.71% to 81.82% for Staphylococcus aureus and Klebsiella aerogenes respectively..

Multiple resistance to antibiotics was observed in all the isolates except among the Salmonella species. The prevalence rates of MAR among the other isolates were: Staphylococcus aureus 25.71%, E. coli 33.33%, Kleb.

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aerogenes, 72.73% and Bacillus species 40.0%. In all, 30(35.29%) of all the potential pathogenic isolates encountered in this study were resistant to two or more antimicrobial agents tested.

Figure 2

Table 2: Profile of Antimicrobial resistance of Potential Human Pathogens isolated from ready-to-eat salads

Resistance profiles	No. of various isolates in each profile					Total no (%)
	S.aureus N=35	E.coli N=21	Kleb aerogenes N=11	Salm.spp N=3	Bacillus spp N=15	
Resistant to 1 antibiotic	0	1	1	2	2	6(7.06)
Resistant to 2 antibiotics	0	2	3	0	1	6(7.06)
Resistant to 3 antibiotics	1	1	0	0	2	4(4.71)
Resistance to 4 antibiotics	1	1	2	0	0	4(4.71)
Resistant to 5 antibiotics	1	0	0	0	3	4(4.71)
Resistant to 6 antibiotics	0	1	2	0	0	3(3.53)
Resistant to >7antibiotics	6	2	1	0	0	9(10.59)
Multiple resistance in Each bacterial species	9(25.71)	7(33.3)	8(72.73)	0(0.0)	6(40.0)	30(35.29)

DISCUSSION

The number of documented outbreaks of human infections associated with the consumption of raw vegetables has increased in recent years [13]. According to the report by the Center for Diseases Control and prevention (CDC) [14], the number of produce related outbreaks per year double between the periods 1973-1987 and 1988-1992. During both periods, the aetiologic agents were known in more than 50% of the outbreaks.

In this study, the objective was focused on the isolation of potential pathogens in ready to eat salads. Surprisingly only five bacterial species were isolated. In a similar study reported by CDC, outbreaks with identified aetiology were predominantly of bacterial origin with *Listeria* spp, *E.coli* serovars, and *Staphylococcus aureus*, *Klebsiella* spp. and *Bacillus cereus* predominating [15]. More recently *Klebsiella* has been linked with infection of tomatoes [16]; *E. coli* infection with lettuce and enterotoxigenic *E. coli* with carrots. Enteric pathogens are among the human pathogens to pose the greatest risks when present on fresh vegetables. This is because of their potential to grow in salads prior to consumption and perhaps have low infective dose as well. In this study the potential pathogens isolated were *S. aureus*, *E. coli*, *Klebsiella aerogenes* *Bacillus* spp. and *Salmonella* *Enterica* var *Typhimurium*. The findings in this study are

partly in agreement with the report of Tony et al., [17] in Taiwan.

The isolation of *Salmonella* species in this study is very disturbing particularly as these samples were obtained from large wedding party receptions. In Nigeria outbreaks following consumption of salads are seldom reported, so there is lack of information on this mechanism of food borne infection transmission.

The high incidence of bacterial contamination of ready to eat salads reported in this study may partly be accounted for by the large numbers of people (usually friends or relations) deployed to assist during weddings, in the preparation of vegetable salads who may not observe basic sanitation requirements for processing produce that requires no pre-heating before consumption. Another reason may be the non-availability of water in good quantity and quality for washing and pre- disinfection of the fresh vegetables and fruits during mass production of salads as in weddings and birthday parties.

The low incidence of bacterial contamination and absence of *Salmonella* spp in salad samples collected from fast food centers is an indication of a higher level of sanitation and personal hygiene observed during the preparation of the salads. The isolation of *S. aureus* and *E. coli* from many of the samples indicate not the preponderance of *S. aureus* on the skin but also the low level of sanitation and personal hygiene as typified by the presence of *E. coli*

The susceptibility tests on the isolates were variable with the highest resistance rate of 81.82% and 25.71% as the lowest rate. An overall multiple resistant rate of 35.29% is reported in this study. This finding probably links the source of these isolates to humans who are commonly colonized by multiple drug resistant bacteria in this geographic region. [18]

It is concluded here that ready to eat salads contain various bacterial species that are potential human pathogens and that these organisms carry multiple drug resistant genes which are transferable to susceptible strains in humans that consume this produce. Due to worldwide emergence of old and new infectious agents and the fact that the world is now a global village, we advocate the establishment and enforcement of National microbiological standards for ready to eat fresh vegetable salads in Nigeria.

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