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Antibiotic resistance pattern of isolated bacterial from salads

Authors:
Adebayo EA, Majolagbe ON, Ola IO and Ogundiran MA.

Institution:
Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria.

Corresponding author:
Adebayo EA.

Email:
brogoke2003@yahoo.com;
olusolanat@yahoo.com;
olaiyabo@yahoo.com;

ABSTRACT:

The antibiotic resistance pattern of bacterial isolates from salad samples sold in a re-known food industry in different parts of Nigeria was investigated. A total of twenty-five bacterial isolates of six genera were encountered in the following proportion: *Pseudomonas* spp. (36%), *Bacillus* spp. (24%); *Escherichia coli* (16%), *Proteus* spp. (12%), *Enterobacter* spp. (8%) and *Aeromonas hydrophila* (4%). The antibiotic resistance pattern of the isolates revealed that resistance to eleven of the twelve antibiotic tested were above 50%. Streptomycin (44%) was the only antibiotic with resistance rate below this range. Resistance to Augmentin was the highest (96%), followed by Cotrimoxazole (92%) and Nitrofurantoin (88%). The results suggest the need for intensive surveillance of isolates throughout salad production continuum to prevent food-borne infections and also to detect emerging antimicrobial resistance phenotypes.

Keywords:
Salads, antibiotic, resistance, pattern and bacterial.

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+2348038099092.

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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 (Uzeh *et al*., 2009). The inner tissues of healthy plants and animals are free of micro-organisms. However, the surfaces of raw vegetables and meats are contaminated with a variety of micro-organisms and this depends on the microbial populations 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 (Pelczer *et al*., 2006).

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 degradation by plant pathogens. These punctures can also promote the multiplication of pathogens especially at non-refrigerated temperatures (Udo *et al*., 2009). During salad preparation, raw vegetables are injured through peeling, slicing or shredding (Beuchart, 2002). These operations can transfer pathogenic micro-organisms 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 absence of spoilage signs, salads are consumed because they are perceived as safe.

The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades (Chiu *et al*., 2002; Threlfall *et al*., 2000), possibly as a result of selection pressure created by the use of antimicrobials of food-producing animals (Angulo *et al*., 2000; Bywater, 2004; Teuber, 2001). The co-existence of resistance genes with mobile elements such as plasmids, transposons, and integrons facilitates the rapid spread of antibiotics resistance genes among bacteria (Sunde and Nordstrom, 2006).

Several investigation have been carried out on the microbial quality of ready to-eat-food, and antibiotic resistance of source human bacterial isolates such as *Salmonella* species (Anh *et al*., 2001; Ehara *et al*., 2004; Nguyen *et al*., 2005). However, the antimicrobial resistant patterns of salad isolated pathogenic organisms have not been studied extensively. This study was therefore carried out to examine antimicrobial resistance pattern of salad isolated pathogenic organisms.

MATERIALS AND METHODS

Samples Collection

The ready-to-eat salads were purchased in a highly respected fast food industry from five different towns of Nigeria, (Ogbomoso, Ado-Ekiti, Ilorin, Ibadan and Lagos). The same were purchased in a sealed container brought to the Laboratory for microbial analysis.

Isolation of organisms

From each salad sample, 10g was aseptically weighed and soaked in 50mls of sterile water for 30mins. Serial dilution was carried out and organisms were plated out on nutrient agar plates using pour plate method. The cultures were incubated at 37^{O}C for 24hrs. After the incubation, the pure cultures of the isolates were obtained by subsequent sub-culturing on fresh agar plates.

Identification of Isolates

The characterization of isolated organisms was done based on cultural, morphological and biochemical standard methods (Buchanan and Gibbons, 1974).

Antibiotic Susceptibility Testing

Susceptibility of each isolate to a panel of twelve antimicrobial substances was assessed by disk diffusion on Mueller-Hinton agar Plates, according to the Clinical and Laboratory Standards Institute guidelines. (CLSI, 2007). The following antimicrobials were tested: Augmentin, AUG, (30µg), ceftriazone, CEF (30µg), Nitrofurantoin, NIT, (20µg), Gentamicin, GEN (10µg), Cotrimoxazole, COT (25µg), Ciprofloxacin, CIP (10µg), Tetracycline, TET (30µg), Erythromycin, ERY (5µg) Streptomycin, STR (10µg), and Chloramphenicol, CLO (30µg). After 18h incubation at 37^{O}C, the size of the zone of inhibition was measured and interpreted by comparing with the standard antibiotic sensitivity chart to determine their resistance patterns.

RESULTS

A total number of 25 bacteria isolates belonging to six genera were identified using biochemical tests for identification as shown in **Table 1.** The distribution of the bacterial isolates in the salad is presented in **Table 2,** with high occurrence obtained in *Pseudomonas* species 9 (36%), followed by *Bacillus* species 6(24%), *E. coli* 4(16%), *Enterobacter* spp. 2 (8%) and *Aeromonas hydrophila* 1 (4%), with the lowest occurrence.

Table 3 presents the number of antibiotics to which resistance was demonstrated. Twenty four isolates, which is the highest 24(96%) were

Table 1: Biochemical characterization of the isolates

CODE	Citrate Unlisation	Motility	Indole Test	Glucose	Frucose	Maltose	Lactose	Sucrose	Galactose	Xylose	Arab	Raff	Rham	Dulc	Mann	Probable Identity
1	-	+	+	+	-	+	+	+	+	-		+	-	+	-	*Bacillus alvei*
2	+	+	-	+	-	+	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
3	-	+	+	+	+	+	+	D	+	D	-	-	D	D	+	*Escherichia coli*
4	+	+	-	+	-	+	+	+	+	-	+	-	-	+	+	*Pseudomonas putida*
5	+	+	-	+	-	+	+	+	+	-	-	-	-	D	+	*Pseudomonas putida*
6	+	+	-	+	-	-	-	+	D	+	-	+	+	-	+	*Pseudomonas florescens*
7	+	+	-	+	-	-	-	+	D	+	-	+	+	-	+	*Escherichia coli*
8	-	+	+	+	+	+	+	D	+	D	-	-	D	D	+	*proteus mirabilis*
9	+	+	-	+	+	+	+	+	+	-	+	-	-	+	+	*Aerononas hydrophila*
10	+	+	-	+	-	-	-	+	+	+	-	-	-	-	-	*Proteus vulgaris*
11	-	+	+	+	+	+	+	D	D	+	-	D	-	-	+	*Proteus vulgaris*
12	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	*Euterobacter aerogenes*
13	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	*Pseudomonas putida*
14	+	+	-	+	+	+	+	+	+	+	-	D	+	+	D	*Bacillus cereus*
15	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	*Bacillus alvei*
16	+	+	-	+	+	+	+	-	+	-	-	-	+	D	-	*Bacillus cereus*
17	-	+	+	+	+	+	+	+	+	-	-	-	+	+	-	*Baccilus alvei*
18	D	+	-	+	+	+	+	-	+	-	-		+	D	-	*Bacillus cereus*
19	-	+	+	+	+	+	+	d	+	D	+		D	D	+	*Escherichia*

Keys:
+ = a positive reaction **- = a negative reaction** **D = a delayed reaction**

Table 2: Distribution and Proportion of bacterial contaminants in salad.

Isolates Identified	Prevalence
Bacillus spp.	6 (24%)
Pseudomonas spp.	9 (36%)
E. coli	4 (16%)
Proteus spp.	3 (12%)
Enterobacter spp.	2 (8%)
Aeromonas hydrohila	1 (4%)

resistant to Augmetin, 23(92%) to Cotrimoxazole, 22(88%) to Nitrofurantoin, 20(80%) to Gentamicin, Amoxicillin and Ciprofloxacin respectively, 19 (76%) to Chloramphenicol, 18(72%) to Tetracyline and Erythromycin, 17(68%) to Ceftriazone and Ofloxacin, and the least 11 (44%) to Streptomycin.

Table 4 show the different resistance pattern observed in the isolated organisms, with the highest numbers obtained at *Bacillus* Spp. (AUG, NIT, CUT, CIP, TET, ERY, CHL).

DISCUSSION

There is a need for continued surveillance of emerging anti-microbial resistant organisms isolated from ready-to-eat food samples, most especially fast foods. This is because there is steadily accruing evidence from around the world, which indicate food as a potential source of antimicrobial-resistant organisms (Schoeder et al., 2004). The results from this study revealed that various salad samples get contaminated with bacteria, which can be highly pathogenic to human.

In the study, *Pseudomons* spp. was the most common bacterial isolate with an occurrence of 36%. *Pseudomonas* spp. when found in food samples indicate ineffective methods of sterilization used during food processing. Since *Pseudomonas* spp. is a prominent inhabitant of soil and water, which can be responsible for diseases of vegetables like angular leaf spot of cucumber and have significance health importance to human beings (Uzeh *et al*., 2009). The organisms isolated in this study are potential pathogens, such as *Pseudomonas* spp., *Bacillus* spp., *E. coli* and *proteus* spp. which partly in agreement with the report of Tony and Que-King (2003) in Taiwan. The high percentage of *E. coli* (16%) isolated is a threat to salad lovers. *E. coli* infection with lettuce and enterotoxigenic *E. coli* with carrots has been established in literature (Udo *et al*., 2009). *E. coli* when found in water and food supplies, is an indicative of a recent faecal contamination and is a threat to public (Mora *et al*., 2005). Its presence is a major health concern,

Table 3: Distribution and proportion of antibiotic resistance among bacterial isolates

Isolates identified	Total number	Total number percentage resistance exhibited to antibiotics										
		Aug	CEF	NIT	GEN	COT	OFL	AMX	CIP	TET	ERY	STR	CLO
Pseudomonas spp.	9	8(89%)	6(67%)	8(89%)	8(89%)	9(100%)	7(79%)	7(79%)	5(56%)	6(67%)	8(89%)	6(67%)	5(56%)
Bacillus spp.	6	6(100%)	5(85%)	6(100%)	5(83%)	6(100%)	3(50%)	5(83%)	6(100%)	6(100%)	5(83%)	4(67%)	5(83%)
E. coli	4	4(100%)	3(75%)	4(100%)	3(75%)	3(75%)	2(50%)	4(100%)	4(100%)	3(75%)	3(75%)	2(50%)	4(100%)
Proteus spp.	3	3(100%)	2(67%)	2(67%)	2(67%)	2(67%)	3(100%)	2(67%)	2(67%)	1(33%)	1(33%)	0	3(100%)
Aeromonas hydrophila	1	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	0	1(100%)	1(100%)	0	0	1(100%)	0
Enterobacter spp	2	2(100%)	0	1(50%)	1(50%)	2(100%)	2(100%)	1(50%)	2(100%)	2(100%)	1(50%)	2(100%)	2(100%)
Total.	25	24(96%)	17(68%)	22(88%)	20(80%)	23(92%)	17(68%)	20(80%)	20(80%)	18(72%)	18(72%)	11(44%)	19(76%)

AUG: Augmetin (30µg), CEF. (Ceftriazone (30µg), NIT: Nitrofurantoin GEN: Gentamicin (10µg), COT: Cotrimoxazole (25 µg), OFL: Ofloxacin (5µg), AMX: Amoxycillin (25µg), CIP: ciprofloxacin (10µg), TET: Tetracyline (30µg), ERY: Erythromycin (5µg), STR: Streptomycin (10µg), CLO: Chloramphenicol (30µg).

Table 4: Antibiotic resistance patterns in isolates identified

Isolates	No of isolates that demonstrated resistance	Antibiotic resistance pattern observed	No of Antibiotic to which resistance was shown
Pseudomonas spp.	1	AUG, CEF, NIT, GEN, COT, AMX, CIP, TET	8
	2	AUG, NIT GEN, COT, OFL CIP, ERY, STR, CHL	9
	1	CEF, NIT, GEN, CUT, OFL, AMX, TET, ERY, CHL	9
	2	AUG, CEF, NIT, COT, OFL, AMX, CIP, TETE, ERY, STR.	10
	2	AUG, CEF, NIT, COT LFL, AMX, TET, ERY,	8
	1	AUG, NIT, GEN, COT, OFL, CIP, TET, ERY	8
Bacillus spp.	2	AUG, CEF, NIT, GEN, COT, AMX, CIP, TET, STR,	9
	3	AUG, NIT COT CIP, TET, ERY, CHL,	7
	1	AUG, CEF, NIT, GEN, COT, OFL, AMX, CIP, TET, ERY, STR, CHL	12
E. coli	1	AUG, CEF, NIT, GEN, COT, AMX, CIP, TET, ERY, CHL,	10
	1	AUG, NIT, GEN, COT, AMX, CIP, TET, CHL,	8
	1	AUG, CEF, IIT, GEN, OFL, AMX, CIP, ERY, CHL	9
	1	AUG, CEF, NIT, COT, OFL, AMX, CIP, TET, ERY, STR	10
Proteus spp	1	AUG, CEF, NIT, GEN, OFL, AMX	6
	1	AUG, COT, OFL, CIP, TET, ERY, STR	7
	1	AUG, CEF, NIT, GEN, COT, AMX, CIP,TET, ERY, CHL	10
Aeromonas hydrophila	1	AUG, CEF, NIT, AMX,	4
Enterobacter spp.	1	AUG, CEF, NIT, AMX, CHL	5
	1	AUG, COT, OFL, AMX, CIP, TET, ERY, STR	8

especially in cases of verotoxin producing *E. coli* (VTEC) serogroup 0157, a major cause of haemorrhagic colitis. Faecal contamination of food cannot be prevented entirely, particularly in this setting where hygienic standard of food production is low and not monitored. Such organisms will continue to be an inhabitant of food for the foreseeable future at least in this part of the world (Oluyege *et al*., 2009).

The high incidence of bacterial contamination of ready to eat salads reported in this study may be accounted for lack of basic sanitation requirements for processing products that requires no pre-heating before consumption. Another reason may be using a low quality of water during washing and pre-disinfection of the fresh vegetables and fruits during salads production (Udo *et al*., 2009).

The antibiotic resistance pattern demonstrated by the bacterial isolates in this study revealed that resistance was highest in all tested antibiotic except Streptomycin (44%). The antibiotic resistance pattern obtained in this study is a serious challenge to public health because of the higher demanding for salads in different homes, societies and functions. In recent years, the number of documented outbreaks of human infections associated with the consumption of raw vegetables has greatly increased (CFS, 2006). The resistant ability of the organisms can be transferred from one organism into another, through the antibiotic resistance plasmids (Olsen *et al*., 2003). Epidemiological data from Huang *et al* (2001) suggests that humans become colonized with antimicrobial-resistant *E. coli* from food consumption. Upon colonization, these organisms may transfer antimicrobial determinants to other bacteria including potential pathogens in the intestinal flora of man (Mizan *et al*., 2002).

Years ago, organisms resistant to multiple drugs were found mostly in hospitals, where antimicrobial agents were used most extensively, but resistance is currently found almost as frequently in the community. The study demonstrated the occurrence of multiple antibiotic resistances among bacterial isolates in a re-known fast food industry in Nigeria. This study therefore emphasizes the need for good hygienic practices, proper handling, storage and retail of salads in a sanitized environment. Also, there is a need for intensive surveillance of isolates throughout the food production continuum to detect emerging antimicrobial resistance phenotypes in developing countries like Nigeria.

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