(RESEARCH ARTICLE)

Identification and antibiotic sensitivity profile of potential bacterial toxin producers from some foods sold to students within Enugu state university of science and technology (ESUT) campus

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Abstract

Food borne diseases have become major public health burden and bacteria especially the toxin producers have been mostly implicated as among the most common causative agents. Thus, the need to screen and identify most frequent bacteria associated with ready to eat foods consumed within ESUT campus. The food samples were chicken, egg, tomato, vegetable salad and dried fish. A total of 25 samples were collected, with 5 for each sample .They were cultured for bacterial isolation using selective media prior to phenotypic characterization via morphology and biochemical analysis . The predominant isolates were further subjected to genotypic characterization using 16S rRNA sequencing. Each identified isolate of inoculum size of 1.5x 108cfu/ml was exposed *in-vitro* to analytical Gram+ve and Gram-ve antibiotics by disc diffusion method .The results revealed the presence of *Staphylococcus aureus strain OPD001-1* in 5samples, *Citrobacter werkmanii* strain UMH18 in 3 samples, *Salmonella enterica subsp. entericaserovar* strain *CFSAN027396* in 4 samples, *Salmonella enterica subsp. entericaserovar typhimurium* in 4 samples and *Salmonella enteric strain 2011k-1440* in 4 samples and *Salmonella enteric subsp. entericaserovar* in 5 samples respectively. Their antibiotic susceptibility pattern differed. Gram positive *Staphylococcus aureus* was susceptible (>20mm) to 7 test antibiotics (Erythromycin, Levofloxacin, Cephalexin, Ciprofloxacin, Gentamicin, Ofloxacin and Clindamycin) and resistant (<14mm) to 3 (Cetriaxone, Ampicillin and Cloxacillin). *Citrobacter werkmanii* and *Salmonella enteric subsp. entericaserovar* demonstrated similar susceptibility (>20mm) to 7 test Gram-ve antibiotics but resistant to Amoxicillin, Streptomycin and Gentamicin while *Salmonella enterica* strain *2011k-1440* and *Salmonella enteric subsp. entericaserovar typhimurium* were resistant (<14mm) to Nitrofurantion , Cetriaxone and Amoxicillin. Although these bacterial isolates can be inhibited by some antibiotics, their implications as potential toxin producers including the heat stable ones pose health problems to food consumers.

Keywords: Foodborne diseases; Bacteria; Antibiotic susceptibility

1. Introduction

Enugu State University Of Science and Technology (ESUT) is located at Agbani in Enugu state, Nigeria. The campus sits on about 500 hectares , housing 9 faculties and about 50,000 students. Despite its massive environment, it lacks a proper eating place like restaurant or cafeteria. Foods are sold in the open like bukas. However, staff and students are compelled to eat from food vendors whose foods are exposed to contamination in the open spaces.

Food is a major source of food illness (both poisoning and intoxication) due to their nutrient composition which are also source of nutrients for microbial growth [1]. Food and food products are always at risk of contamination through various means including pre and post harvest, processing, handling, transportation, storage and unhygienic preparations [2].

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Microorganisms especially bacteria play a prominent role in food deterioration with subsequent toxin production, more worrisome are the microorganisms that are present in food without any effect on the food but are toxin producers. Bacterial pathogens example *Salmonella*, *Staphylococcus* are responsible for a wide range of human infections including food intoxication [3]. Food borne diseases have become a major public health problem as they result in mortality, morbidity and economic loss [4]. Consumption of foods contaminated with microorganisms may result in infections that affect the cardiovascular, muscular, skeletal, respiratory and immune systems [5].

Molecular characterization of microorganisms is important in identification because it is used in detection of genetic variation in microorganisms as well as epidemiologic analysis of infectious microorganisms and public health surveillance. The aim of this study was to isolate and identify potential bacterial toxin producers from some foods sold to students within ESUT campus.

2. Material and methods

Samples were collected for a period of 5 weeks. A total of 25 food samples (5 samples each week) from ready to eat chicken, eggs, tomatoes, vegetable salad and dried fish were bought from different food vendors within ESUT campus. All samples were wrapped separately in sterile transparent wide-mouthed bottles and transported to the laboratory for microbial analysis.

2.1. Preparation of stock solution of the food samples

Ten grams (10 g) of each sample was weighed int0 5ml sterile water and agitated to obtain stock solution of the sample after which 1 ml of the sample was inoculated into already sterilized 9ml peptone water (Merck, Germany) and incubated at 37 ^{0}C for 24h for enrichment [6].

2.2. Isolation of bacteria from food samples

Each enriched sample (0.1 ml) was cultured on Mannitol salt agar (Himedia, India) and Salmonella Shigella agar (Himedia,India) plates already prepared according to manufacturers specifications and incubated at 37 ^{0}C for 24 h.The growths were subcultured into fresh SSA and Mannitol agar plates to obtain discrete pure colonies. Each colony was subcultured into fresh nutrient agar plates (TM media, India) re-incubated at 37 ^{0}C for identification purposes [1,6].

2.3. Identification

2.3.1. Phenotypic identification

Identification commenced with colonial appearances on the plate, motility test, gram staining and some biochemical tests including oxidase, catalase, coagulase, indole, H_2S production, methyl red and glucose fermentation [6, 4].

2.3.2. Genomic identification

The predominant isolates were subjected to genomic identification. The DNA isolation and other procedures were adopted as described by [7].

2.3.3. Antibiotic susceptibility test

Antimicrobial susceptibility testing by disc diffusion method as described by [8] was adopted. A gram +ve antibiotic disc (Polydisc) containing Erythromycin, Cetriaxone, Ampicillin, Cloxacillin, Cephalexin, Ciprofloxacin,Gentamicin, Ofloxacin and Clindamycin was used for *Staph aureus* strain while gram-ve antibiotic disc (Poly disc) containing Nitrofurantion, Gentamicin,Ciprofloxacin, Chloramphenicol, Meropenem, Pefloxacin, Cetriaxone, Amoxicillin and Streptomycin was used for *Salmonella* and *Citrobacter* strains. The bacterial innocula were prepared from a 24h culture. A loopful each of the young culture was diluted in sterile water to obtain a density comparable to 0.5 macfarland standard turbidity scale corresponding to about 1.5 x 10^8 Cfu/ml. One millimeter each of the bacterial suspension was spread on the surface of Muller Hinton agar (MHA) for 10min. Thereafter antibiotic disc were firmly placed and incubated at 37 ^{0}C for 24 h. The resulting zones of inhibition were measured with ruler. The obtained results were compared with the recommended values for resistance and susceptibility [9].

3. Results

Table 1 Phenotypic identification scheme for the Bacterial Isolates

S/N	Isolate code	Colony Appearance	Gram Stanning	Motility Test	Catalase	Coagulase	Oxidase	H$_2$S Production	Indole	Methyl red	Glucose fermentation	Lactose fermentation	Suspected genera
1	ST1	Small sized opaque yellow colonies	+ve cocci in clusters	_	+	+	_	_	_	+	+	+	*Staph aureus*
2	ST2	Small sized opaque colonies	+ve cocci in clusters	_	+	_	_	+	_	_	+	+	*Staph epidermis*
3	ST3	Small sized opaque yellow colonies	+ve cocci in clusters	_	+	+	_	_	_	+	+	+	*Staph aureus*
4	SC1	Black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
5	SC2	Black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
6	MVS1	Black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
7	MVS2	Opaque colonies	-ve rod	_	+		_	_	_	+	+	_	*Shigella spp*
8	MDF1	Smooth black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
9	MDF2	Opaque colonies	-ve rod	_	+		_	_	_	+	+	_	*Shigella spp*

10	MDF3	Black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
11	SE1	Smooth black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
12	SE2	Smooth black centered colonies	-ve rod	+	+		_	+	_	+	+	_	*Salmonella spp*
13	MT1	Medium size yellow colonies	-ve rod	+	+		_	+	_	+	+	+	*Citrobacter spp*
14	MT2	Medium size yellow colonies	-ve rod	+	+		_	+	_	+	+	+	*Citrobacter spp*
15	MT3	Small sized opaque colonies	+ve cocci in clusters	_	+	+	_	_	_	+	+	+	*Staph aureus*

Table 2 Molecular Characterization Of Bacterial Isolates

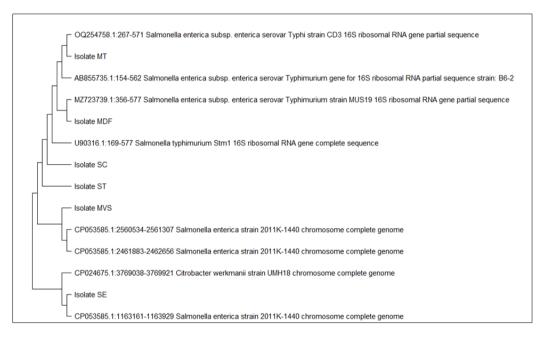| Isolate code	Species/strain	%Similarity	Genebank accession number	Basepair
ST	*Staphylococcus aureus* Strain *OPD001-1*	71 .26%	CP 121234.1	636206-636937
MT	*Citrobacter werkmanii* Strain *UMH18*	91.03%	CP024675.1	3769038- 3769921
SC	*Salmonella enteric susbp. entericaserovar strain CFSAN027396*	78.33%	CP74245.1	448926-4490028
MVS	*Salmonella enterica* strain *2011K-1440*	89.22%	CP05585.1	2560534- 2561307
MDF	*Salmonella enterica subsp entericaserovar typhimurium strain B6-2*	78.66%	AB855735.1	154-562
SE	*Salmonella enterica* strain*2011k – 1440*	89.22%	CP053585.1	1163161-1163929

Figure 1 Phylogenetic scheme of bacterial isolates

Table 3 Recovery Pattern Of Predominant Bacteria From The Food Samples

Identified Bacteria	Type of Food Samples Present					Number of Times Recovered	%Recovery
	T	E	DF	VS	C		
S. aureus strain *OPD001-1*	✓					5	20
C. werkmanii Strain *UMH18*	✓					3	12
S.enterica subsp entericaserovar strain *CFSAN027396*		✓				4	20
S. enterica subsp. entericaserovar typhimurium			✓			4	16
S. enterica strain *2011K-1440*				✓		4	20
S. enterica subsp entericaserovar					✓	5	20
TOTAL						25	100

T= Tomatoes, E = Eggs, DF= Dried fish. VS= Vegetable salad, C= Chicken

Table 4 Antibiotics Susceptibility Pattern Of Food Bacterial Isolates

Bacterial Isolates	Zones of Inhibition (mm)									
	Gram –ve Antibiotics									
	N100	GN10	CIP10	C10	MP10	PF10	CT30	AX30	ST30	
C. werkmanii	26	11	30	28	22	21	24	12	10	
S. enterica subsp. Strain *entericaserovar CFSAN02739 6*	20	13	24	30	24	26	25	10	8	
S. enterica subsp. entericaserovar typhimurium	11	25	23	28	21	24	13	12	24	
S. enterica strain *2011K-1440*	10	22	24	26	23	22	11	10	20	
	Gram +veAntibiotics									
	E10	CT30	AM30	CL10	LV5	CX30	CIP5	GN10	OF10	CD10
S. aureus strain *OPD001-1*	22	10	12	8	20	24	26	20	20	22

Key: Gram –ve antibiotics: N100= Nitrofurantion(100mcg), GN10= Gentamicin(10mg), CIP10=Ciprofloxacin(10mg), C10=Chloramphenicol(10mcg),MP10=Meropenem(10mcg), PF10=Pefloxacin (10mcg), CT30=Cetriaxone (30mcg),AX30=Amoxicillin (30mcg),, ST=Streptomycin(30mcg); Gram+ve antibiotics: E10=Erythromycin(10mcg),CT30=Cetriaxone(30mcg),AM30=Ampicillin(30mcg), CIP5= Ciprofloxacin (5mcg), GN10= Gentamicin(10mcg).

4. Discussion

In the present study, 25 samples of 5 different food items were collected and analyzed. Table 1 and table 2 shows the morphological characterization and biochemical tests which suggested the isolates to be *Staphylococcus spp*, *Citrobacter spp*, and *Salmonella spp*. The genomic characterization further confirmed the isolates to be the suspected organisms. They were identified to be *Staphylococcus aureus* strain *OPD001-1*, *Ctrobacter werkmanii* strain *UMH18*, *Salmonella enteric subsp. entericaserovar* strain *CFSAN027396*, *Salmonella enteric* strain *2011k-1440*, *Salmonella enterica subsp. entericaserovar typhimurium* respectively.

Table 3 shows the percentage occurrence of the organisms with *Staphylococcus aureus* as the bacteria with the highest frequency occurrence of 32%. This could be due to the fact that *S. aureus* is abundant in the human body especially skin as a normal flora, the food substances may have been contaminated during handling. The prevalence of *S. aureus* in many food products especially vegetables is an indicator that consumers are at risk of infection by *S. aureus* and possibly enteric diseases. This also conforms with the findings of other researchers [10]. The percentage of *Salmonella* isolates were 56% in which the predominant species were *Salmonella enterica subsp. entericaserovar* strain *CFSAN027396* 20% and *Salmonella enterica strain 2011-1440* 20% followed by *Salmonella enterica subsp .entericaserovar typhimurium* 16%. *Salmonella spp* is one of the most important food borne pathogens worldwide. In recent times, there has been reports of increased food borne salmonellosis in various countries which includes Spain, Italy, Thailand, England and America. These outbreaks are linked to a variety of foods like poultry meat, eggs, fish, diary products [11].The least predominant bacteria was *Cirtrobacter werkmanii* with 12% occurrence. This may be as a result of poor hygiene, sanitary conditions in the environment or disease spreading vectors such as cockroaches and flies [12]. *Citrobacter werkmanii* is also usually considered a food poisoning bacteria [13]. However, the occurrence of these pathogenic organisms is a major concern for public health [14].

Table 4 and 5 shows the antibiotic susceptibility pattern of the isolates. *Staphylococcus aureus* strain was susceptible to Erythromycin 10mcg, Levofloxacin 5mcg, Cephalexin30mcg, Ciprofloxacin 5mcg, Gentamicin10mcg, Ofloxacin 10mcg and Clindamycin 10mcg but resistant to Cextriaxone 30mcg, 30mcg and Cloxacillin 30mcg. This is in accordance with a similar report where 90% sensitivity was observed in bacterial isolates from street foods [15].*Citrobacter werkamii* and *Salmonella enterica subsp. entericaserovar* demonstrated similar susceptibility with zones of inhibition (<20mm) to 6 test Gram –ve antibiotics (Nitrofurantion 100mcg, Ciprofloxacin 10mcg. Chloramphenicol 10mcg, Meropenem 10mcg, Pefloxacin 10mcg, Cetriaxone 30mcg) but resistant to Amoxicillin, Streptomycin and Gentamicin. *Salmonella enterica strain 2011k- 1440* and *Salmonella enteric subsp. entericaserovar typhimurium* were resistant (<14mm) to Nitrofurantion 100mcg, Cetriaxone 30 mcg and Amoxicillin 30 mcg. There is an increasing report of prevalence of Amoxicillin-Clavulanate resistance due to continous spread of β-lactamase mediated resistance [6]. The resistance of

Salmonella strain against Nitrofurantion , a broad spectrum antibiotic used for the treatment of uncomplicated urinary tract infection (UTI) is in line with the findings of [16]. However, the majority of the bacterial isolates showed maximum sensitivity against the tested antibiotics.

5. Conclusion

This study reveals the prevalence of pathogenic bacteria that maybe potential toxin producers in food samples which pose a threat to consumers. Some of the food samples analyzed are eaten raw while some are normally cooked before consumption which may not be appropriate for the elimination of the pathogenic bacteria. This however highlights the need for the prevention of contamination of foods to ensure food safety.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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