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RESEARCH ARTICLE

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Occurrence and diversity of *Vibrios* on lettuce irrigated with wastewater in Kano

Dahiru M.[1]* and Enabulele O. I.[2]

[1]*Department of Biological Sciences, Faculty of Sciences, Federal University Kashere, Gombe State, Nigeria*
[2]*Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria*
Correspondence: musahanifa@yahoo.com

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ABSTRACT

Pathogenic Vibrios has emerged as a serious and global threat to human health. The research investigates occurrences and diversity of Vibrios species on lettuce and wastewater from Jakara canal that is used to irrigate it.Samples culturedby incubation in alkaline peptone water (APW) and cultured on thiosulfate-citrate-bile-sucrose (TCBS; Merck) agar. Pigmented colonies were Grams' stained, tested for oxidase, catalase, motility, and indole, cholera red, salt tolerance in (0%, 3%, 6%, 8% and 10% (NaCl) and glucose, sucrose, lactose and arabinose fermentation tests and antibiotics susceptibility profiles were determined. V. cholerae was detected in 11.27% of samples, while V. paraheamolyticus was detected in 12.68%, V. damsel was much less, had 1.41% all of lettuce samples. Similarly, V. fluvialis and V. mimicus were detected each in 2.82% of wastewater. Vibrios species were detected much higher on lettuce than wastewater but no statistical significance (P > 0.05) was observed.Fifty percent (50.00%) of V. cholrae detected from lettuce were resistant to pefloxacin and while V. fluvialis, V. hollisae and V. mimicus were more resistant to nalidixic acid 66.66%, 43.86% and 28.57% respectively. Resistant by V. vulnificus, V. fluvialis, V mimicus and V. paraheamolyticus to ampicilin (33.33%, 33.33%, 25.00% and 16.66% respectively) was also observed. The antibiotic susceptibility patterns were quite similar, and showed statistical significance, at both (P < 0.05) and (P < 0.01) probability levels. Multi-drug resistance was equally observed among all isolates. Theresult demonstrates diverse Vibrio species present in lettuce and wastewater was resistant to antibiotics that should be sensitive to, traditionally. The diversity and resistant patterns were quite a challenge to clinical and public health in future control of Vibrio specie targeted approach.

Key words: pathogenic *Vibrio*, irrigation wastewater, lettuce, multi- drugs resistance, Jakara canal.

INTRODUCTION

Several studies throughout the world have demonstrated a very close relation between the consumption of fruits and vegetables irrigated with raw wastewater and many food borne diseases like gastroenteritis, cholera, chemical toxicity etc[1]. The World Health Organization estimates, 200, 000 deaths from food borne pathogens in Nigeria [2].

Of particular concerned in developing countries is the cholera outbreak, which have continue be a life straitening disease. Pathogenic *Vibrios* have emerged as a serious and global threat to human health. The incidence of infections has risen sharply worldwide with the appearance of pandemic clones of greater infective ability [3]. Here in Nigeria, epidemiological data from Public Health Department of Kano State Ministry of Health, revealed that the frequency and distribution of cholera epidemics in the State during 1995 to 2001, were 2,630 in 1995/1996, 847 in 1997 and 2,

347 in 1999 [4]. Many of the sources that were thought to contribute to the epidemiology of disease were raw fruits and vegetables, which were equally impacted by ecological conditions that affect survival or growth of pathogenic microorganisms [5]. These sources include raw manure, inorganic amendments, irrigation water and dust and therefore the microbial quality of a vegetable grown with wastewater shall remain a high public health priority [6].

Although cholera is primarily known as a water-borne disease in endemic regions however, contamination of foods can also be an imperative mode for cholera transmission. In Nigeria cholera is highly prevalent in northern part of the country (due to poor sanitation), where many outbreaks has been implicated to the consumption of faecally-contaminated foods and water [7] [8] [9]. Usually, large-scale outbreaks cause a high burden of disease and rapidly overwhelm preventive and curative health care services, particularly where public health systems have broken down [10]. In addition to this, large-scale cholera outbreaks cause great economic loss as inappropriate external restrictions may lead to disruptions in trade and travel and Kano being center of commerce the outcomes is usually disastrous.

In the midst of meningitis outbreak [11]of 1996, Kano state (1996 population: 4,931,789) was struck by another large-scale outbreak of severe diarrhea that was soon confirmed to be cholera[7]. Most outbreaks were often not caused by a single common source but rather by the ingestion of various types of foods or water that become contaminated through various unidentified breaks in hygienic practices [10] [12]. Hutin, *et al*. [7] argue that, estimating the proportion of cases that could be prevented through implementation of selected improved hygienic practices is a better approach than attempting to identify outbreak-specific vehicles. However according to this research, identify any specific vehicle for these types outbreaks could serve as critical control point in the prevention of the disease.In Kano state, there were few microbiologically based survey of *Vibrio* abundance and diversity detectedin general as well as specifically suspected available food has been carried out. Along these lines, the current study attempt to find out the level of contamination by *Vibrio* (abundance and diversity) especially lettuce grown with contaminated wastewater from Jakara canal, as well as determined their antibiotic susceptibility response to the commonly used antibiotics, with a view to demonstrate the public health risk of consuming it and drug of choice for the treatment diseases cause by *Vibrios* species.

MATERIALS AND METHODS

Sampling procedure

Sample sites were chosen based on three different points along the canal and the farms around each point. The first point stated from Airport road down to Kaura-goje, the second is located at Gayawa village, while the last was Wase Dam (i.e. the terminal point of the canal). Water and lettuce (*Crassostreavirginica*) samples were collected on the same day approximately every one week during May to July 2012 from the Jakara canal and lettuce irrigated with canal wastewater. Wastewater samples were collected in sterile bottles on site by filling and then capping the 1 Liter bottles at ~30 cm below the water surface, while lettuce were collected in a sterile polyethylene bag and transported to laboratory for the analyses.

Bacteriological Analysis.

Enrichment for potentially pathogenic*Vibrio* species was performedin alkaline peptone water. All enriched samples werecultured on thiosulfate-citrate-bile-sucrose (TCBS; Merck) agar plates with 2% NaCl and incubated at 37°C for 18 to 24 h. Pigmented colonies on TCBS (yellow and green) were subjected to Grams' stained, salt tolerancein (0%, 3%, 6%, 8% and 10% (NaCl) and sugar fermentation (glucose, sucrose, lactose and arabinose) cytochrome oxidase, catalase activity, motility, indole and cholera red test were conducted for specie identification [13] [14]. All Vibrio positive colonies were subjected to antibiotic susceptibility test using ten commonly used antibioticson standard antibiotic discs (G-VE, Polytes Laboratories, Enugu, Nigeria) it includes ampicillin (PN) 30 µg, ofloxacin (OFX) 10 µg, streptomycin (S) 30 µg, ceporex (CEP) 10 µg, cotrimoxazole (SXT) 30 µg, gentamicin (CN) 10 µg, nalidixic acid (NA) 30 µg, amoxicillin clavulanate (AU) 30 µg, ciprofloxacin (CPX) 10 µg and pefloxacin (PEF) 10 µg, susceptibility patterns were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria [15]. A Spearman rank correlation andT test were used forstatistical analyses.

RESULTS

Isolation of bacteria

V. cholerae and six other species were detected during the study (Table 1). *V. paraheamolyticus* was detected most frequently, in 12.68% of all samples, specifically all *V. paraheamolyticus* were detected from lettuces samples, similar high frequencies of detection was also observed *V. cholarae* (11.27% of all samples) with 12% and 9.52% in lettuce and wastewater samples respectively. *V. damsela* was detected with much less frequently (1.41% of all samples), only in lettuce. Similarly *V. fluvialis* and *V. mimicus* were also less frequently detected (2.82% of all samples), werehowever detected only in wastewater. Although highest number of the *Vibrios* species were detected from lettuce, but there was no statistical significance (P > 0.05) in percentage prevalence between lettuce and wastewater.

Table 1.Percentage occurrence and diversity of *Vibrio*on lettuce and wastewater sampled from Jakara wastewater canal and irrigation farms in Kano

S/N	Bacterial Isolates	No (%) on Lettuce n= 50	No (%) in Wastewater n= 21	Total No. Species
1	*V. cholera*	6(12.00)	2(9.52)	8(11.27)
2	*V. vulnificus*	00	2(9.52)	2(2.82)
3	*V. fluvialis*	00	2(9.52)	2(2.82)
4	*V. mimicus*	5(10.00)	2(9.52)	7(9.86)
5	*V. parahemolyticus*	9(18.00)	0(0.00)	9(12.68)
6	*V. hollisae*	5(10.00)	0(0.00)	5(7.04)
7	*V. damsel*	1(2.00)	0(0.00)	1(1.41)
	Total	26(52.00)	8(38.10)	34(47.89)

Key: n = number

Table 2.Percentage prevalence of antibiotic resistance patterns of *Vibrios* species detected on Lettuce sample

			Resistance Prevalence (%)											
Taxons	N	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	R^2	R^3	R^4
V. Cholera	6	00	116.16	00	00	33.33	50.00	16.66	00	00	00	00	16.66	00
V. mimicus	14	7.14	28.57	35.71	14.28	28.57	14.28	28.57	21.43	14.28	14.28	14.28	21.42	14.28
V. Parahaem	13	00	23.08	23.08	15.38	23.08	15.38	7.69	7.69	7.69	00	7.69	15.38	15.38
V. hollisae	14	00	14.28	14.28	21.43	42.86	28.57	14.28	14.28	21.43	7.14	21.43	14.28	21.43
V. demsela	7	00	14.28	28.57	00	14.28	14.28	42.86	28.57	14.28	00	14.29	14.29	14.29
V. fluvialis	3	00	00	33.33	66.66	66.66	00	00	33.33	66.66	66.66	33.33	00	66.66

Key: N = Total number of isolates. OFX = Ofloxacin, PEF = Peflacine, CPX = Ciproflox, AU= Amoxicillin-clavulanate, CN = Gentamycine, S = Streptomycine, CEP = Ceporex, NA = Nalidixic acid, SXT = Co-trimethezole, PN = Ampicillin, Parahaem = Parahaemolyticus

Table 3.Percentage prevalence of antibiotic resistance pattern of *Vibrios* species detected from Jakara Wastewater

Taxons						Resistance Prevalence (%)								
	N	S	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	R^2	R^3	R^4
V. cholera	6	16.66	33.33	16.66	00	50.00	00	16.66	16.66	16.66	16.00	16.66	16.66	16.66
V. mimicus	4	25.00	25.00	00	25.00	25.00	00	00	25.00	25.00	50.00	25.00	50.00	00
V. Parahaemolyticus	6	16.66	16.66	00	16.66	00	00	00	33.33	16.66	00	16.66	00	16.66
V. fluvialis	6	00	33.33	00	16.66	00	16.66	16.66	16.66	00	16.66	00	16.66	00
V. vulnificus	6	16.66	33.33	33.33	00	16.66	33.33	33.33	00	00	00	16.66	00	33.33

Key: N = Total number of isolates. OFX = Ofloxacin, PEF = Pefloxacin, CPX = Ciprofloxacin, AU= Amoxicillin-clavulanate, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Co-trimotxazole, PN = Ampicillin, R^2 = resistant to 1 -2 drugs, R^3 = resistant to 3 drugs, R^4 resistant to 4 drugs and above

Antibiogram profile

Fifty percent (50.00%) of *V. cholrae* detected from lettuce were resistant to pefloxacin and sensitive to ceporex, amoxicillin-clavulanate, ciprofloxacin and co-trimethazole. Other *Vibrios: V. fluvialis, V. hollisae* and *V. mimicus* were more resistant to nalidixic acid at 66.66%, 43.86% and 28.57% respectively. However, susceptibility pattern in *V. cholrae* detected from wastewater was not similar to that of lettuce isolates, more resistance of nalidixic acid and sensitive to pefloxacin was observed instead. Resistant by *V. vulnificus, V. fluvialis, V mimicus* and *V. paraheamolyticus* to ampicillin (33.33%, 33.33%, 25.00% and 16.66% respectively) was also observed. In addition, multi-drug resistance species from both sources was observed, and higher in species detected from lettuce. *V. fluvialis* was highest (66% resistance to 4 drugs and above), followed by *V. hollisae* and *V. cholerae* which

demonstrate the least multi- drug resistant among species from lettuce. *V. mimicus* specie from wastewater had 50% multiple drug resistance to 3 drugs.

Table 4. Correlation matrix of percentage prevalence of antibiotic resistance patterns of *Vibrios* species detected on Lettuce sample

	S	PN	CEP	OFX	NA	PEP	CN	AU	CPX	SXT	R2	R3	R4
S	1												
PN	-.048	1											
CEP	.482	-.798	1										
OFX	-.106	-.538	.450	1									
NA	-.166	-.207	.080	.899*	1								
PEP	-.176	.884*	-.937**	-.643	-.259	1							
CN	.327	.044	.146	-.703	-.752	.101	1						
AU	.150	-.769	.829*	.568	.317	-.841*	.173	1					
CPX	-.134	-.599	.520	.957**	.843*	-.694	-.494	.748	1				
SXT	-.008	-.433	.486	.956**	.875*	-.624	-.553	.656	.965**	1			
R2	-.038	-.782	.604	.862*	.726	-.736	-.323	.817*	.932**	.831*	1		
R3	.527	.413	-.234	-.858*	-.782	.508	.579	-.548	-.899*	-.847*	-.751	1	
R4	-.165	-.631	.538	.972**	.823*	-.733	-.555	.717	.991**	.954**	.914*	-.909*	1

Keys: = 0.05 level, **0.01 level, OFX = Ofloxacin, PEF = Pefloxacin, CPX = Ciprofloxacin, AU= Amoxicillin-clavulanate, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Co-trimotxazole, PN = Ampicillin, R^2 = resistant to 1 -2 drugs, R^3 = resistant to 3 drugs, R^4 resistant to 4 drugs and above*

Table 5. Correlation matrix of percentage prevalence of antibiotic resistance patterns of *Vibrios* species detected from Jakara canal wastewater sample

	S	PN	CEP	OFX	NA	PEP	CN	AU	CPX	SXT	R2	R3	R4
S	1												
PN	-.408	1											
CEP	.153	.562	1										
OFX	.069	-.583	-.875	1									
NA	.477	.404	.381	-.479	1								
PEP	-.357	.562	.688	-.458	-.292	1							
CN	-.327	.802	.869	-.802	.144	.869	1						
AU	.185	-.829	-.867	.704	-.217	-.867	-.967**	1					
CPX	.749	-.583	-.458	.445	.419	-.875	-.802	.704	1				
SXT	.372	-.005	-.460	.617	.316	-.451	-.490	.276	.605	1			
R2	1.000**	-.408	.153	.069	.477	-.357	-.327	.185	.749	.372	1		
R3	.373	.000	-.456	.609	.328	-.456	-.488	.275	.609	1.000**	.373	1	
R4	.218	.134	.869	-.802	.144	.535	.643	-.564	-.357	-.734	.218	-.732	1

*Keys: *= 0.05 level, ** 0.01 level, OFX = Ofloxacin, PEF = Pefloxacin, CPX = Ciprofloxacin, AU= Amoxicillin-clavulanate, CN = Gentamycin, S = Streptomycin, CEP = Ceporex, NA = Nalidixic acid, SXT = Co-trimotxazole, PN = Ampicillin, R^2 = resistant to 1 -2 drugs, R^3 = resistant to 3 drugs, R^4 = resistant to 4 drugs and above*

The correlation analyses of antibiotic susceptibilities patterns of *Vibrios* species detected from lettuce on antibiotics were quite similar, and showed statistical significance, for example PEP/PN and PEP/OFX were similar at 88.4% ($P < 0.05$) and 93.7% ($P < 0.01$). Other significant correlations observed were NA/OFX 89.9 ($P < 0.05$), CPX/OFX 95.7% ($P < 0.01$), AU/CEP 82.9% ($P < 0.05$) and CPX/NA 84.3% ($P < 0.05$). However, the response were not the same in the case of *Vibrio* detected from wastewater, the result demonstrated no correlation in the susceptibility patterns between antibiotic tested, the only significance correlation was negative -96.7% ($P < 0.01$), nevertheless all *Vibrios* resistant to co-trimithazole, significantly correlated to group of *Vibrio* exhibiting resistance to three antibiotics ($P < 0.01$) and streptomycine to those resistance to two antibiotics ($P < 0.01$).

DISCUSSION

Considering percentage of *V. cholerae* and other species previously implicated in gastrointestinal problems, lettuce grown in Kano, could serve as source of transmission of cholera to public especially during outbreaks. It is often reported outbreaks Kano state were not caused by a single common source but rather by the ingestion of various types of food and beverages that become contaminated through various unidentified breaks in hygienic practices [10] [12] not limiting to environmental sanitation and direct link between contamination sources and their vehicles. Therefore detection of *V. cholerae* is a clear demonstration of the possibility of lettuce involvement in the transmission as previously speculated. Similarly, the detection of *V. cholerae* in water (wastewater) had further demonstrated therole of water as one of the vehicle mostly implicated for transmission. While drinking water sold in the streets by water vendors was associated with illness, whose sources of contamination are varied and determined, the source of contamination of wastewater by *Vibrios* may be obvious. In a most recent survey, [16] on coliforms contamination of households' drinking water in some parts of Kano Metropolis, Nigeria reported 83.0% contamination with coliform bacteria and similarly, water contaminated during storage has also been associatedwith the spread of cholera in India [17] and in Peru [18], elsewhere drinking of street-vended water has been implicated with cholera outbreaks in Latin America [12]. It is quite possible the lettuce might be contaminated through the wastewater, but the diversity of species detected on the lettuce demonstrates not only as candidate for *V. cholerae* but for other gastrointestinal pathogenic species like *V. paraheamolyticus* and *V. vulnificus.* Ingestion of seafood is the most common mode of transmission for *V. parahaemolyticus* as evidenced by numerous outbreaks reported from different parts of the world where people regularly consume improperly processed and undercooked seafood [19]. With the current growing global importance in *V. parahaemolyticus* infections [20] this finding, have therefore highlighted a significant public health point of attention. Tunung, *et al*, [21] had detected *V. paraheamolyticus* from raw salad vegetables (lettuce samples were 16) at retail level in Selangor, Malaysia and therefore concluded that raw vegetables could be contaminated with virulent *V. parahaemolyticus* and could act as a transmission route, thus poses risk to consumers from the consumption of raw vegetables. Susceptibility patterns demonstrated by *Vibrios* detected in this work have poses a great public health risk of overlapping ecological niche.

The *Vibrios* detected in this work had showed a wide and varied level of resistant to a number of antibiotics epically pefloxacin, nalidixic acid and ampicilin .In general, wastewater *Vibrios* were not different from lettuce *Vibrios* in their responses to these antimicrobial agents, and thus inferring their capability of transferring antimicrobial resistance genes among themselves and to other bacteria. This is demonstrated in the apparent overlap between waste (contaminant) from various sources to wastewater canal, including hospital, abattoirs, domestic and industrial waste and the lettuce. Previously, Ole, and his colleagues in 2009 point that spread of antimicrobial resistance is not necessarily restricted by phylogenetic, geographic, or ecological borders. Thus, use of antimicrobial agents in one ecological niche, may impact the occurrence of antimicrobial resistance in other ecological niches [22].

Multi-drug resistance demonstrated by isolates ranged from 2 to 4 was high with all the pathogenic species involved is alarming. Most studies on the antimicrobial susceptibility profiles of *Vibrio* species focus almost exclusively on clinical and/or food isolates [23] with little information on isolated from environmental sources such as irrigation wastewater and pre-harvest lettuce. To our knowledge, this is the first study that specifically investigatesoccurrence diversity and antimicrobial susceptibility profile and detection of multiple antibiotics resistance of *Vibrio* strains isolated from municipal wastewater used for irrigation and the irrigated vegetable in Kano Nigeria.

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