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Incidence of Acinetobacter in Fresh Carrot (*Daucus carota* subsp. *sativus*)

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Abstract**—**The research aims to investigate the occurrence of multidrug-resistant Acinetobacter, in carrot and estimate the role of carrot in its transmission in a rapidly growing urban population. Thus, 50 carrot samples were collected from Jakara wastewater irrigation farms and are analyzed on MacConkey agar and screened by Microbact 24E (Oxoid) and susceptibility of isolates is tested against 10 commonly used antibiotics. *Acinetobacter baumannii* and *A. lwoffii* were isolated in 22.00% and 16% of samples respectively. Resistance to ceporex and penicillin of 36.36% and 27.27% in *A. baumannii*, and sensitivity to ofloxacin, pefloxacin, gentimycin and co-trimoxazole were observed. However, for *A. lwoffii* apart from 37.50% resistance to ceporex, it was also resistant to all other drugs tested. There were similarities in the resistances shown by *A. baumannii* and *A. lwoffii* to fluoroquinolones and β- lactame drug families in addition to between sulfonamide and animoglycoside demonstrated by *A. lwoffii*. Significant correlation in similarities were observed at $P < 0.05$ to CPX to NA (46.2%), and SXT to AU (52.6%) *A. baumannii* and *A. lwoffii* respectively and high multi drug resistance (MDR) of 27.27% and 62.50% by *A. baumannii* and *A. lwoffii* respectively. The occurrence of multidrug-resistance pathogen in carrot is a serious challenge to public health care, especially in a rapidly growing urban population where subsistence agriculture contributes greatly to urban livelihood and source of vegetables.

Keywords**—**Urban agriculture, Public health, Fluoroquinolone, Sulfonamide, Multidrug-resistance.

I. Introduction

THE growing urban population due to influx of rural dwellers to cities has led to increased urban food demand, which encourages subsistence agricultural practice in vacant plots and other available land in urban and peri-urban settlements, to fill the gap of food supply and for livelihood and food security reasons. This has effects on both food safety and transmission of zoonotic pathogens. Food safety is a concept relating to handling, preparation, and storage of food in ways that prevent food borne illnesses from production to consumption known as the "farm-to-plate" or "stable-to-table" concepts. It has, been shown that consumption of vegetables poses a greater risk for public health compared to handling cattle or drinking milk [1]. To this effect, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin [2]. As a whole, leafy green vegetables were cited as a source of 26% of the food-borne outbreaks in United States, between 1998- 1999 [3]. Food is not only nutritious to humans, but also an ideal breeding ground for bacteria. Such pathogens may be *Brucella* in unpasteurized milk [4], *Salmonella* spp. shed by pigs and faecal coliforms on vegetables [5].

For vegetables, there are several sources of microbial contamination in the production chain. Levels of fecal coliforms in water used for irrigation often exceed the WHO wastewater irrigation guidelines [5]-[7]. In humans, *Acinetobacter* can colonize skin, wounds, and the respiratory and gastrointestinal tracts. Some strains of *Acinetobacter* can survive environmental desiccation for weeks, a characteristic that promotes transmission through fomite contamination in hospitals [8], [9]. Most alarming is the organism's ability to accumulate diverse mechanisms of resistance, the emergence of strains that are resistant to all commercially available antibiotics [10] and the lack of new antimicrobial agents in development to control the bacteria [11]. Multidrug- resistant *Acinetobacter* was isolated from 20% of wounds and from blood and respiratory secretions [12]. *A. baumannii* was the most prevalent nosocomial pathogen reported in a Turkish ICU in which casualties of the 1999 Marmara earthquake were treated [13].

Acinetobacter during the past three decades has emerged from an organism of questionable pathogenicity to an infectious agent of importance to hospitals worldwide [8], [14], [15]. The main challenge with *A. baumannii* is the ability to acquire antimicrobial-resistance genes extremely rapidly, leading to multidrug resistance [16]. Thus, occurrence of *Acinetobacter* has become a public health challenge not only in clinical management but also in a population with low socio economic power. In an attempt to track the presence and determine the potential risk to human, of transmission of multidrug-resistance pathogens, the research aimed to isolate *Acinetobatcer* species in carrot.

II. Material and Methods

The study was carried out in Jakara canal wastewater irrigation farms. The major sources of water supply to Jakara canal are wastewater from domestic houses, laundries, Kano main Abattoir, and hospitals. The water collected into the canal is drained to Wase Dam, as its collection terminal. Farmers use the water to grow vegetables, starting immediately from any vacant plot and continue along the canal neighboring farms, till its final point of collection. Fifty samples of carrot were randomly collected from different farms aseptically, and transported to laboratory for analyses. All samples were initially processed to separate the non-fermenters from other Gram-negative bacilli on MacConkey

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agar at 37°C for 24 hours. Samples were sub cultured from primary isolation media and grown further on nutrient agar (NA), from which colonies on NA were Gram stain, other biochemical tests conducted include oxidase, catalase gelatin liquefaction, motility and other sugar fermentation. These were done in accordance with Microbact 24E (Oxoid) for the identification of unknown oxidase negative bacteria, incubated at 37°C and 44°C after inoculation on Microbact strips. Antimicrobial susceptibility tests using disc diffusion method was carried out [17] with Ofloxacin (OFX), Pefloxacin (PEF), Ciproflox (CPX), Amoxicillin-clavulanic acid (AU), Gentamycin (CN), Streptomycin (S), Ceporex (CEP), Nalidixic acid (NA), Co-trimoxazole (SXT), Ampicilin (PN) and results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria [18].

TABLE I
PERCENTAGE DISTRIBUTION OF *ACINETOBACTER* SPECIES ON CARROT OCCURRENCE/RESISTANCE PROFILES ON COMMONLY USED ANTIBIOTICS

Sources (n=50)	No. Isolated	% Occurrence	% Percentage Resistance of Antibiotic									
			ST	PN	CEP	OFX	NA	PEP	CN	AU	CPX	SXT
A.baumannii	11	22.00	9.09	27.27	36.36	0.00	18.18	0.00	0.00	18.18	9.09	0.00
A. lwoffii	8	16.00	0.00	12.50	37.50	25.00	12.50	12.50	12.50	25.00	12.50	12.50

Key: n = Total number of sample, % = percentage, ST = Streptomycin, PN = Ampicilin, CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin- clavulanic acid, CPX = Ciproflox, SXT = Co-rimoxazole.

TABLE II
ANTIBIOTICS SUSCEPTIBILITY PHENOTYPES SIMILARITIES OF *ACINETOBACTER* SPECIES ISOLATED ON CARROT

Antibiotics	ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
S	1									
PN	.414	1								
CEP	.015	.488*	1							
OFX	-.180	-.146	.123	1						
NA	-.319	.439	.366	-.055	1					
PEF	-.097	-.240	.136	-.129	-.062	1				
CN	.108	.014	-.400	.101	.109	.032	1			
AU	.035	.062	.316	-.286	.281	.424	.150	1		
CPX	-.104	.531*	.016	-.029	.462*	.034	.403	.210	1	
SXT	.260	-.156	-.131	-.238	.189	.077	.426	.526*	-.017	1

Key: *. = (p) 0.05, ST = Streptomycin, PN = Ampicilin, CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin- clavulanic acid, CPX = Ciproflox, SXT = Co-trimoxazole,.

TABLE III
ANTIBIOTICS SUSCEPTIBILITY PHENOTYPES SIMILARITIES OF *ACINETOBACTER BAUMANNII* AND *A. IWOFFII* ISOLATED ON CARROT

	Antibiotics	ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	
	ST	1	.134	-.452	-.463	.286	-.051	.603	.365	-.058	.778*	
	PN	.429	1	-.213	.194	.454	-.186	.641	-.363	.809*	-.203	
	CEP	.132	.819**	1	.450	-.046	.366	-.440	.346	-.499	-.096	
	OFX	.127	-.326	-.262	1	.055	-.440	-.196	-.545	-.045	-.381	
Acinetobacter baumannii	**NA**	-.417	.472	.534	-.224	1	-.295	.812*	.285	.471	.469	***Acinetobacter lwoffii***
	PEF	-.077	-.248	-.097	.500	.058	1	-.279	.517	-.271	-.153	
	CN	-.010	-.330	-.359	.653*	-.265	.596	1	.164	.635	.485	
	AU	-.063	.456	.337	.084	.345	.195	.051	1	-.394	.686	
	CPX	-.153	.422	.372	.075	.495	.502	.216	.900**	1	-.290	
	SXT	.091	-.130	-.196	.081	.077	.740**	.263	.156	.433	1	

Key: *. (p) 0.05, **.(p) 0.01, ST = Streptomycin, PN = Ampicilin, CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin- clavulanic acid, CPX = Ciproflox, SXT = Co-trimoxazole.

III. RESULTS

Two species of *Acinetobacter* were isolated and *A. baumannii* occurred most with 22.00% from the samples than *A. lwoffii* which had 16.00% occurrence, as shown in Table I. The result of antibiotics susceptibility test on the isolates also demonstrate resistance phenotype, with the high percentage of 36.36% resistant to ceporex and 27.27% penicillin by *A. baumannii*, however, ofloxacin, pefloxacin, gentimycin and co-trimpxazole were sensitive to it. Similarly, majority of *A. lwoffii* isolates were also resistant to ceporex (37.50%) and resistant to other drugs that demonstrated sensitivity to *A. baumannii* (ofloxacin, pefloxacin, gentimycin and co-trimoxazole) that had percentage resistance ranged from 12.50% to 25.00% (Table I). In summary 36.84% of isolate from all samples were more resistant to ceporex and penicillin (21.05%). There were some similarities in the resistant phenotype exhibited by both *A. baumannii* and *A. lwoffii* at $P < 0.05$ confidence limit. For example, CPX to NA, PN to CEP, SXT to AU and CPX to PN were 46.2%, 48.8%, 52.6%, and 53.1% respectively as shown in Table II. However, the result was not the same (Table III), when the resistant profiles of the species were compared separately. *A. baumannii* showed

similarity in CPX to AU (90%, P < 0.01) and CEP to PN (81.90%, P < 0.01), while *A. lwoffii* demonstrate resistant similarities in SXT to S, CPX to PN, CN to NA with 77.8%, 80.90% and 81.20% all at P < 0.05, respectively.

Although, certain percentage of both *A. baumannii* and *A. lwoffii* were resistance to either of the drugs tested, 9(47.37%) did not demonstrated resistance to any of the sensitive drugs above, 6(54.55%) *A. baumannii* 3(37.50%) *A. lwoffii*). Multi drug resistance (MDR) phenotype was observed, with overall MDR of 8(42.11%), of these 3(27.27%) were *A. baumannii* and 5(62.50%) were *A. lwoffii* as shown in Table IV.

TABLE IV
DISTRIBUTION OF *ACINETOBACTER BAUMANNII* ISOLATE ON CARROT AND MULTIDRUG RESISTANCE PROFILES

Specie Isolated	Antibiotics										% Multidrug Resistance
	ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	
A. baumannii (n = 11)	S	S	R	S	S	S	S	S	S	S	**1(10.00)**
	R	R	R	S	S	S	S	S	S	S	**3(30.00)**
	S	R	R	S	R	S	S	S	S	S	**3(30.00)**
	S	R	R	S	R	S	S	R	R	S	**5(50.00)**
	S	S	S	S	S	S	S	R	S	S	**1(10.00)**
% total	9.09	27.27	36.36	00	18.18	00	00	18.18	9.09	00	
A. lwoffii (n = 8)	S	S	R	R	S	S	S	S	S	S	**2(20.00)**
	S	R	S	S	R	S	R	S	R	S	**4(40.00)**
	S	S	S	S	S	S	S	R	S	R	**2(20.00)**
	S	S	R	S	S	R	S	R	S	S	**3(30.50)**
	S	S	R	R	S	S	S	S	S	S	**2(20.00)**
% Total	00	12.5	33.5	25.00	12.5	12.5	12.5	25.00	12.5	12.5	

Key: % = percentage, ST = Streptomycin, PN = Ampicilin, CEP = Ceporex, OFX = Ofloxacin, NA = Nalidixic acid, PEF = Pefloxacin, CN = Gentamycin, AU= Amoxicillin- clavulanic acid, CPX = Ciproflox, SXT = Co-trimoxazole, S = Sensitive, R= Resistance.

IV. DISCUSSION

The research recorded higher percentage occurrence of *Acinetobacter baumannii* on carrot samples from irrigation farms in Kano, than *A. lwoffii*. The occurrence of known nosocomial and multidrug-resistance pathogen in carrot that are mostly consumed raw is a challenge to public health care, especially with the growing urban populations and rapid increased in urban food production, that have demonstrable effects on both food safety and transmission of zoonotic pathogens. In as much as the influx of rural dwellers (who are mostly low income earners) to cities continues, and dwellers maintain parts of their agriculture practices using untreated wastewater, the risk of transmission of pathogenic bacteria will continue to be priority public health issues in developing countries. This is supported by the occurrence of *Acinetobacter* in water and soil, and it's isolated from foods, arthropods, and the environment, as reported by [14]. The report of *Acinetobacter* colonizing human skin, wounds, and the respiratory and gastrointestinal tracts indicate further the possible risk of transmission infection by this pathogens, through vegetables to human [8], [9]. Elsewhere, multidrug-resistant *Acinetobacter* was only reported isolated from wounds, blood, and respiratory secretions [12], others reported *Acinetobacter* as most prevalent nosocomial pathogen in a Turkish intensive care unit (ICU) where casualties of the 1999 Marmara earthquake were treated [13]. It is evident that the high need of water has necessitates the search for alternative water source (like wastewater) for urban agriculture, the absence of which may drive small-scale farmers into in adequate food supply or even to poverty level, as there is little access to other water sources [19]. Usually, fecal coliforms in water used for irrigation often exceed the WHO wastewater irrigation guidelines [5], [6] and presence of zoonotic pathogens, such as *Salmonella* spp. [7].

A high resistance to ceporex that id positive correlated with penicillin was demonstrated by *Acinetobacter* spp. isolated from this work, although samples not from hospital environment, this observation did not coincide with [20], that the production of AmpC β-lactamases chromosomally encoded cephalosporinases as one of the resistance mechanism usually demonstrated by *Acinetobater* spp. Similarly, *Acinetobacter* spp. were reported to exhibit resistance to quinolones, tetracyclines, chloramphenicol, disinfectants, and tigecycline [21]-[23] through efflux pumps active expel activity (decreased outer membrane permeability) and alterations in the quinolone enzymatic targets (DNA gyrase), ofloxacin, pefloxacin, gentimycin and co-trimoxazole were observed to be sensitive to *Acinetobacter baumannii* and insensitive to *Acinetobacter lwoffii*. This selective sensitivity may be attributed to research error or to low level accumulation of several bacterial mutations (DNA gyrase and bacterial permeability) that was reported to results in the development of resistance to even more effective fluoroquinolones [24]. Resistance to ofloxacin, nalidixic acid, pefloxacin and ciprofloxacin is quit alarming, and challenge to public health care system in Nigeria, because these are among the effective commonly used available fluoroquinolones. To support this Vila and his colleagues [25] had reported only relatively few other antibiotics were reported effective against *Acinetobacter baumannii* unfortunately recent date had reported increased in clinical incidence of fluoroquinolone resistance [26].

Multi drug resistance (MDR) phenotype demonstrated by these species, of *A. baumannii* and *A. lwoffii* have supported the WHO report (2014) that resistance to common bacteria has

reached alarming levels in many parts of the world indicating that many of the available treatment options for common infections in some settings are becoming ineffective. In Africa, the information concerning the true extent of the problem of AMR is limited because surveillance of drug resistance is carried out in only a few countries. These bring about scarcity of accurate and reliable data on AMR, for many common and serious infectious conditions that are important for public health [27].

REFERENCES

[1] Grace, D., Olowoye, J., Dipeolu, M., Odebode, S., and Randolph, T. "The influence of gender and group membership on food safety: the case of meat sellers in Bodija market, Ibadan, Nigeria. Tropical Animal Health and Production" 44 *Suppl* 1, S53-9. 11250-012-0207-0, 2012.

[2] Berger, N. C., Samir, V. S., Robert, K. S., Griffin, P. M., David, P., Paul, H. and Gad, F. "Fresh fruit and vegetables as vehicles for the transmission of human pathogens" *Environ. Microbiol.* 12(9), 2385–2397, 2010.

[3] FAO/WHO "Risk profile for enteroheamorrhagic *Escherichia coli* including the Identification of the Commodities of Concern, including Sprouts, Ground Beef and Pork" *CX/FH 03/5/Add*.4 Sept. 2002. Codex alimentarious commission, 2003.

[4] Makita, K., Desissa, F., Teklu, A., Zewde, G., and Grace, D. "Risk assessment of staphylococcal poisoning due to consumption of informally-marketed milk and home-made yoghurt in Debre Zeit, Ethiopia". *International Journal of Food Microbiology*, 153(1-2), 135-41, 2012.

[5] Amoah, P., Drechsel, P., Henseler, M. and Abaidoo, R. C. "Irrigated urban vegetable production in Ghana: microbiological contamination in farms and markets and associated consumer risk groups" *Journal of Water Health*. 5, 455-66, 2007.

[6] World Health Organization "Guidelines for the safe use of wastewater, excreta and greywater" 2006. Retrieved from, www.who.int/water_sanitation_health/wastewater/gsuww/en/

[7] Ndiaye, M. L., Dieng, Y., Niang, S., Pfeifer, H. R., Tonolla, M., and Peduzzi, R. "Effect of irrigation water on the incidence of Salmonella spp. on lettuces produced by urban agriculture and sold on the markets in Dakar, Senegal". *African Journal of Microbiology Research*, 5(19), 2885-2890, 2011.

[8] Schreckenberger P. C., Daneshvar M. I., Weyant R. S., and Hollis D. G. "Acinetobacter, Achromobacter, Chryseobacterium, Moraxella, and other nonfermentative gramnegative rods" In: Murray P. R., Baron E. J., .Jorgensen J. H., Landry M. L., Pfaller M. A., eds. Manual of clinical microbiology. 9th ed. Washington, DC: ASM Press, 2007:770- 802.

[9] Getchell-White S. I., Donowitz L. G., and Groschel D. H. "The inanimate environment of an intensive care unit as a potential source of nosocomial baceria: evidence for long survival of Acinetobacter calcoaceticus" *Infect Control Hosp Epidemiol*, Vol. 10:402-7, 1989.

[10] Lolans K., Rice T. W., Munoz-Price L. S., and Quinn J. P. "Multicity outbreak of carbapenem-resistant Acinetobacter baumannii isolates producing the carbapenemase OXA-40" *Antimicrob Agents Chemother*, 50:2941-5, 2006.

[11] Talbot G. H., Bradley J., Edwards J. E. Jr, Gilbert D., Scheld M., and Bartlett J. G. "Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America" *Clin Infect Dis.* 42:657-68, 2006.

[12] Silvia M. L. and Weinstein, R.A. "*Acinetobacter* Infection" *N Engl J Med*; 358:1271-81, 2008.

[13] Oncul O, Keskin O, Acar HV, *et al*. "Hospital-acquired infections following the 1999 Marmara earthquake" *J Hosp Infect*, 51:47-51, 2002.

[14] Fournier, P. E. and Richet, H. "The epidemiology and control of *Acinetobacter baumannii* in health care facilities" *Clin. Infect. Dis*. 42:692-699, 2006.

[15] Dima S., Kritsotakis E. I., Roumbelaki M., et al. "Device-associated nosocomial infection rates in intensive care units in Greece" *Infect Control Hosp Epidemiol*, 28: 602-5, 2007.

[16] Imperi, F., Antunes, L. C., Blom, J., Villa, L., Iacono, M., Visca, P., and Carattoli, A. "The genomics of *Acinetobacter baumannii*: insights into genome plasticity, antimicrobial resistance and pathogenicity" *IUBMB Life*. Dec; 63(12), 1068-74, 2011.

[17] Cheesbrough, M. (2005). District Laboratory practice for tropical countries, Part 2, Cambridge University Press, UK, 426 pp.

[18] Clinical and Laboratory Standards Institute (CLSI) (2007). Performance Standards for Antimicrobial Susceptibility Test Approved Standard (Document M100-S17). 27 (1). 187 pp.

[19] Stine, S. W., Song, I., Choi, C. Y., and Gerba, C. P. "Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce" *Journal of Food Protection*, 68, 913-8, 2005.

[20] Poirel L., and Nordmann P. "Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology" *Clin Microbiol Infect*; 12:826-36, 2006.

[21] Peleg A. Y., Adams J, and Paterson D. L. "Tigecycline efflux as a mechanism for nonsusceptibility in *Acinetobacter baumannii*" *Antimicrob Agents Chemother*; 51: 2065-9, 2007.

[22] Jacoby GA, and Munoz-Price LS. "The new beta-lactamases" *N Engl J Med*; 352:380-91, 2005.

[23] Qi C, Malczynski M, Parker M, and Scheetz MH. "Characterization of genetic diversity of carbapenem-resistant *Acinetobacter baumannii* clinical strains collected from 2004 to 2007" *Clin Microbiol*; *46* : 1106-9, 2008.

[24] Acar, J. F. and Goldstein, F. W. "Trends in bacterial resistance to fluoroquinolones" *Clin Infect Dis.* 1997; 24 (suppl 1): S67–73, 1997.

[25] Vila, J., Ruiz, J., Goni, P., Marcos, A., and Jimenez De Anta, T. "Mutation in the *gyrA* Gene of Quinolone-Resistant Clinical Isolates of *Acinetobacter baumannii" Antimicrob Agents Chemother* 39 (5): 1201–1203, 1995.

[26] Chopra S., Torres-Ortiz M., Hokama L. *et al*. "Repurposing FDA-approved drugs to combat drug-resistant *Acinetobacter baumannii*" *J Antimicrob Chemother*; 65: 2598–601, 2010.

[27] World Health Organization. "Antimicrobial resistance: global report on surveillance" 2014. WHO Press, World Health Organization, 20 Avenue Appia, ISBN 978 92 4 156474 8, 3 – 5 or www.who.int.