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Acinetobacter baumannii in Birds' Feces: A Public Health Threat to Vegetables and Irrigation Farmers

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Abstract

The rising trend of resistance in *Acintobacter baumannii* had in recent days become a public health care concern with most literature reported from samples collected from hospital environment. This research therefore, wishes to determine the occurrence of multidrug-resistant *A. baumannii* in birds' droppings, associated with irrigated farms vegetables, for epidemiological update and future clinical forecast. Forty eight birds fecal samples were collected and processed for isolation and identification of *A. baumannii* on MacConkey agar and Microbact 24E (Oxoid), and tested against 10 commonly used antibiotics (quinolones, fluoroquinolones, aminoglycosides, sulfonamides). *A. baumannii* was isolated from 31.25% of samples and had shown more resistant to ceporex (100.00%) and to streptomycin with 80.00% and 90.00% for Jakara and Sharada farms' fecal samples respectively; isolates were however sensitive to co-trimoxazole. Forty eight (46.67%) of the isolates were resistant to at least 6 drugs, with strong correlation between some drugs. By this result, wild birds' fecal materials demonstrate high potential of *A. baumannii* carrying capacity and dissemination, and thus pose risk of contaminating vegetables, infecting human and transmitting resistance phenotype to other non-multidrug-resistant bacteria—a situation quite challenging to health care management and public health. And thus it further suggests for screening of additional probable contributing factors, so as to develop possible detailed transmission pathway and control strategies.

Keywords

Wild Birds, Vegetables, Public Health, Ceporex, Co-Trimoxazole

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1. Introduction

Acinetobacter species are usually commensal organisms, but they occasionally cause infections, predominantly in susceptible patients in hospitals. They are opportunistic pathogens that may cause urinary tract infections, pneumonia, bacteraemia, secondary meningitis and wound infections. These diseases are predisposed by factors such as malignancy, burns, major surgery and weakened immune systems, in neonates and elderly individuals. There is no evidence of gastrointestinal infection through ingestion of *Acinetobacter* spp. The emergence and rapid spread of multidrug resistant *A. calcoaceticus baumannii* complex, causing nosocomial infections, are of concern in health care facilities. *A. baumannii* is a prevalent species that causes epidemic outbreaks of nosocomial *Acinetobacter* infections [1]. *A. baumannii* is occasionally isolated from environmental samples such as soil and water.

The exact natural habitat of many of the *Acinetobacter* species is yet to be fully understood and may require intense efforts to identify [2]. Thus, overall diversity of habitat, predilection to accumulate antimicrobial resistance, resistant to desiccation, ability to form biofilm, and propensity to cause hospital infection outbreaks make *Acinetobacter* a remarkable microorganism. *A. baumannii* strains are generally more resistant than other species of this genus and often express a multi-drugs resistant (MDR) phenotype. Therefore, treatment of nosocomial infections caused by *A. baumannii* has become complicated because of the widespread antimicrobial resistance among these organisms [3]. The rising trend of resistance in *A. baumannii* strains, particularly to newer antimicrobial agents, has therefore become a public health care concern. The organism expresses multiple mechanisms of antibiotic resistant that likely leads to the development of multiply resistant or even "pan-resistant" strains.

Some authors have suggested hospital environment as the major reservoir of the multidrug resistant *A. baumannii* [4] [5]. It still remained apparent that *A. baumannii* has been isolated from quit a number of other non-hospital sources. For example, Byrne-Bailey *et al.* in [6] had isolated multidrug resistant *A. baumannii* in England from soil fertilized with pig manure, and similarly Zhang and his colleagues in [7] had also isolated multi-drugs resistant *A. baumannii* from livestock in China. Many bacteria were reported as part of the normal intestinal flora of birds. Bird species associating with potentially contaminated environments, such as refuse dumps, sewage treatment facilities, agricultural sites, and bird feeders, are likely to harbor pathogenic bacteria in their intestinal tracts [8]. For example, Craven *et al.* [9] implied that house sparrows and European starlings may be responsible for transmitting *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* to chickens on poultry farms. Ahmed *et al.* [10] have isolated non-multi-drug resistant *A. baumannii* from birds' feces kept in Zoo, Japan. Thus, birds may inadvertently ingest bacteria in their environment, and the bacteria pass through the intestinal tract with no adverse effects to the carrier bird; yet in such cases the birds may aid in dispersal of the bacteria within the environment. The research therefore aims at determining the prevalence of multidrug resistant *A. baumannii* in birds' droppings (carrier rate), especially those associated with vegetables that are minimally process and consumed, in Kano State, and thus, accesses the potential role of wild birds in transmission of *A. baumannii* for public health importance. In Africa, there is paucity of data on the prevalence of *A. baumannii* carried in the fecal samples of domestics or wild birds feeding in irrigation farms in Nigeria; most data on multidrug resistant *A. baumannii* were from Europe and Asia. Therefore an attempt to document these in Nigeria will surely complement the efforts made, for epidemiological and clinical forecast.

2. Material and Methods

The study was carried out in 2 irrigation sites, with the first site located along Jakara wastewater canal irrigation farms and the second site located along Sharada canal wastewater irrigation farms, both located in Kano City. All samples were initially processed to separate the non-fermenters from other Gram negative bacilli on MacConkey agar and 5% sheep blood agar at 37˚C for 24 hours. There after identification was done to confirm presence of *Acinetobacter* spp. 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 were undertaken. These were done in accordance with Microbact 24E (Oxoid) for the identification of unknown oxidase negative bacteria.

Species differentiation on Microbact 24E was done on basis of 24 reactions, and inoculates at 37˚C and 44˚C. Antimicrobial susceptibility tests using disc diffusion method was carried out in accordance with [11] using the following antibiotics, Ofloxacin (OFX), Pefloxacin (PEF), Ciproflox (CPX), Amoxicillin-clavulanic acid (AU), Gentamycin (CN), Streptomycin (ST), Ceporex (CEP), Nalidixic acid (NA), Co-trimoxazole (SXT), Ampicilin

(PN). The results were interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria [12].

Spearman correlation analysis was used to compare the similarities in response to each antibiotic tested on *A. baumannii* isolated

3. Results

From a total of 48 fecal samples, 24 from each site, 5 (20.83%) *Acinetobactaer baumannii* isolates were obtained from fecal samples collected along Jakara irrigation farms, while 10 (41.67%) were obtained from fecal samples collected from Sharada irrigation farms. The overall distribution of antibiotic resistant, for *Acinetobacter* isolates, detected from each site are shown in **Table 1**. Isolates from all sources were more resistant to ceporex (100.00%) followed by streptomycin with 80.00% and 90.00% resistance phenotype in isolates from Jakara farms and Sharada farms fecal samples respectively. All isolates were less resistant to co-trimoxazole with 20.00% and 50.00% in fecal samples from Jakara and Sharada farms respectively. Multi dug resistant of two or more antibiotics was observed among all isolates, with the highest seen in isolates from fecal sample collected in Jakara, for example number JK16 was resistant to all drugs tested. Impact, forty six (46.67%) of the isolates were resistant to at least 6 antibiotics, as showed in **Table 2**. Although, the isolates showed considerable multi drugs resistance, there were however few correlation in the patterns of resistant demonstrated by isolates on each antibiotic. For example only three positive values were observed, which were CEP to PN (67.8%, $p < 0.01$), CPX to

Table 1. Percentage distribution of *Acinetobacter baumannii* occurrence in birds' feces and antibiotic resistant profiles.

Sources	No. sampled	No. Isolated	% Occurrence	Percentage Resistant of Antibiotic									
				ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
Jakara Farms	24	5	20.83	80:00	80:00	100:00	80:00	60:00	100:00	60:00	80:00	40:00	20:00
Sharada Farms	24	10	41.67	90:00	60:00	100:00	70:00	40:00	70:00	60:00	70:00	80:00	50:00

Key: No = Total number, % = 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.

Table 2. Distribution of *Acinetobacter baumannii* isolate from birds' feces and multidrug resistant profiles.

S/N	Sample number	Source	Name of antibiotic										% Multidrug resistance
			ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT	
1	JK 5	Jakara farms	R	R	R	R	S	R	S	R	S	S	60:00
2	JK 8		S	S	R	R	S	R	R	R	S	S	50:00
3	JK 9		R	R	R	R	R	R	R	R	R	S	90:00
4	JK 16		R	R	R	R	R	R	R	R	R	R	100:00
5	JK 20		R	R	R	S	R	R	S	S	S	S	50:00
6	SH 11	Sharada farms	R	S	R	S	S	R	R	R	R	S	60:00
7	SH 12		S	S	R	S	R	R	R	R	S	R	60:00
8	SH 13		R	R	R	R	S	R	S	S	R	S	60:00
9	SH 14		R	R	R	R	R	R	R	R	S	R	90:00
10	SH 17		R	S	R	R	R	S	R	S	R	S	60:00
11	SH 18		R	R	R	S	S	R	S	R	R	R	70:00
12	SH 19		R	R	R	R	S	R	R	R	R	S	80:00
13	SH 20		R	R	R	R	R	R	S	R	R	R	90:00
14	SH 21		R	S	R	R	S	S	S	R	R	R	60:00
15	SH 23		R	R	R	R	S	S	R	S	R	S	60:00
	Column Total		86:67	66:67	100:00	73:33	46:67	80:00	60:00	73:33	66:67	40:00	

Key: OFX = % = 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 = Resistant.

ST (57.6%, $p < 0.05$), AU to SXT and (55.1%, $p < 0.05$) as shown in **Table 3**.

4. Discussion

There have been limited data on the occurrence of *Acinetobatcer baumannii* especially in association with agricultural produce. *A. baumannii* was reported to multiply not only on human and animal skin, but also in soil and water and thus has a diversity of reservoirs [1]. Our present finding is clearly in support of this hypothesis, as demonstrated by the high occurrence of *Acinetobatcer baumannii* isolated from birds' fecal samples. Although other researchers [6] [7] have demonstrated the isolation of *Acinetobatcer* species from some livestock, there was no report of isolation from wild birds' fecal sample. Previously, report has demonstrated bacteria as part of the normal intestinal flora of birds, and therefore bird species associating with potentially contaminated environments, such as refuse dumps, sewage treatment facilities, agricultural sites, and bird feeders, are likely to harbor pathogenic bacteria in their intestinal tracts [8].

The report of Ahmed *et al.* [10] on isolation of non-multi-drug resistant *A. baumannii* from birds' feces kept in Zoo, Japan, does not really demonstrate isolation from wild bird; since the birds were in captivity, this report is more of prevalence or occurrence of *A. baumannii* in wild birds, whose date were previously scarce for *A. baumannii*. Previously birds have been implicated in transmission of pathogenic bacteria; for example, Craven *et al.* [9] implicated house sparrows and European starlings for transmission of *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* to chickens on poultry farms. This suggests that wild birds' fecal samples at farms environment could disseminate multi-drug resistance *A. baumannii* and the possible risk of contaminating and infecting human population is likely, through the consumption of minimally processed fresh vegetables. This could be supported by previously researches, for example Solomon and his colleagues [13] had demonstrated the ability of *E. coli* 0157:H7 to enter lettuce plant through the root system and migrate throughout the edible portion of the plant and thus *A. baumannii* could be expected to have this ability as bacterium; however a more specific research is required to confirm that. Enterobacteriacea were isolated with increasing frequency from fresh produce, including beans, sprouts, cantaloupes, apples, lettuce, [13] and carrot, [14]. In the area of this study Dahiru *et al.* [15] isolated *E. coli* Ol57:H7 in cabbage and lettuce leaves; Uzeh and Adepoju [16] had also reported isolation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* from different salad vegetables: cucumber, cabbage, carrot, and lettuce. A number of reports on contamination of vegetables and other agents of transmission have been documented in [13] [14]; as a whole, leafy green vegetables were cited as a source of 26% of the food-borne outbreaks in United States, between 1998 and 1999 [17].

Table 3. Antibiotic resistant similarities of isolates to the antibiotics tested.

	ST	PN	CEP	OFX	NA	PEF	CN	AU	CPX	SXT
S										
PN	0.342									
CEP	0.310	0.678**								
OFX	0.318	0.059	0.261							
NA	0.234	0.213	0.193	−0.252						
PEF	0.086	0.397	0.026	−0.102	0.020					
CN	0.074	0.068	0.236	0.354	0.006	−0.513				
AU	−0.309	0.086	0.100	−0.233	0.170	0.388	−0.001			
CPX	0.576*	−0.034	−0.077	0.258	−0.241	−0.152	0.337	−0.218		
SXT	−0.040	0.275	0.344	−0.326	0.469	0.165	0.069	0.551*	−0.389	

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.

While birds scavenging in irrigation farms poses risk of contaminating the environment and even the vegetables with bacteria, *A. baumannii* remained to be a challenge to public health, especially due to its pronounce multidrug-resistance mechanisms. In this research birds' fecal samples have demonstrated a high resistance profile to most antibiotics commonly used, not only resistance per say, but a multi-drug or pan drug resistance as demonstrated by some species isolated. Most alarmingly all isolates were resistance to more than one drug, with quite an amount of resistant to at least five drugs. Although this phenomenon was not new in *A. baumannii*, the isolation from birds' fecal sample called for close monitoring and surveillance, so as to address the possible occurrence and rapid transmission, of multi-drug resistance phenotype not only within the genus but also among other Enterobacteriaceae in farm environments. The use of fecal materials or dung of goats, sheep, donkey, birds and many other domestic and wild animals as a source of nutrients to crops is well established practice in Nigeria; this habit contributes in directly amplification of the spread of multi-drug resistance *A. baumannii* and other pathogenic bacteria, even to environments which were never reported to inhabit.

The observation of high resistance by *A. baumannii* isolated from this work to ceporex (β-lactam drug) and streptomycin (aminoglycoside drug), is in harmony with what was previously reported on *Acinetobacter* species, which were shown to exhibit different mechanisms of resistance to antibiotics, including the capability to produce enzymes modifying aminoglycosides, β-lactamases of a wide spectrum of activity, carbapenemases, as well as mechanisms resulting from the changes in outer membrane proteins, in penicillin binding proteins and in topoisomerases [18]. These lead to the formation of multidrug resistant (MDR) strains, and even the pandrug-resistant (PDR) strains which are resistant to all available drugs [19].

The correlation analysis of the resistance phenotype by *A. baumannii* on the ten antibiotics has demonstrated high positive relationship between ceporex and penicillin (both β-lactam); however cotrimothazole which was more sensitive than other drugs has also demonstrated more than fifty percent positive correlation with amoxicillin-clavulanate. These were in agreement with Bonomo and Szabo, who reported resistance mechanisms that are expressed frequently by *Acinetobacter* including β-lactamases, alterations in cell-wall channels (poring), and efflux pumps. *A. baumannii* can become resistant to quinolones through mutations in the genes *gyrA* and *parC* and can become resistant to aminoglycosides by expressing aminoglycoside-modifying enzymes [20], and thus the exhibition of MDR by greater percentage of *A. baumannii* is isolated on this work. The presence study marks the beginning of understanding the importance of multi-drug resistance phenotype by *A. baumannii* in agricultural produce. The occurrence or spillover of multi-drug resistance by wild birds suggests a rather new pathway of transmission that may demand further research input, so as to properly suggest best ways and practice in the control and prevention of diseases caused by *A. baumannii* and other *Acinetobacter* species.

References

[1] Joshi, S.G. and Litake, G.M. (2013) *Acinetobacter baumannii*: An Emerging Pathogenic Threat to Public Health. *World Journal of Clinical Infectious Diseases*, **3**, 25-36. http://dx.doi.org/10.5495/wjcid.v3.i3.25

[2] Visca, P., Seifert, H. and Towner, K.J. (2001) Acinetobacter Infection—An Emerging Threat to Human Health. *IUBMB Life*, **63**, 1048-1054. http://dx.doi.org/10.1002/iub.534

[3] Seifert, H., Baginski, R., Schulze, A. and Pulverer, G. (1993) Antimicrobial Susceptibility of *Acinetobacter* Species. *Antimicrobial Agents and Chemotherapy*, **37**, 750-753. http://dx.doi.org/10.1128/AAC.37.4.750

[4] Dijkshoorn, L., Nemec, A. and Seifert, H. (2007) An Increasing Threat in Hospitals: Multidrug-Resistant *Acinetobacter baumannii*. *Nature Reviews Microbiology*, **5**, 939-951. http://dx.doi.org/10.1038/nrmicro1789

[5] Gootz, D.T. and Marra, A. (2008) *Acinetobacter baumannii*: An Emerging Multidrug-Resistant Treat. *Expert Review of Anti-Infective Therapy*, **6**, 309-325. http://dx.doi.org/10.1586/14787210.6.3.309

[6] Byrne-Bailey, K.G., Gaze, W.H., Kay, P., Boxal, A.B., Hawkey, P.M. and Wellington, E.M. (2009) Prevalence of Sulfonamide Resistance Genes in Bacterial Isolates from Manured Agricultural Soil and Pigs Slurry in the Unoited Kingdom. *Antimicrobial Agents and Chemotherapy*, **53**, 696-702. http://dx.doi.org/10.1128/AAC.00652-07

[7] Zhang, W.J., Lu, Z., Schwartz, S., Zhang, R.M., Wang, X.M., Si, W., Yu, S., Chen, L., and Liu, S. (2013) Complete Sequence of Bla (NDM-1) Carrying Plasmid pNDM-AB from *Acinetobacter baumannii* of Food Animal Origin. *Journal of Antimicrobial Chemotherapy*, **68**, 1681-1682. http://dx.doi.org/10.1093/jac/dkt066

[8] Casanovas, L., Desinion, M., Ferrer, M.D., Arques, J. and Monzon, G. (1995) Intestinal Carriage of Campylobacters, Salmonellas, Yersinias and Listerias in Pigeons in the City of Barcelona. *Journal of Applied Bacteriology*, **78**, 11-13. http://dx.doi.org/10.1111/j.1365-2672.1995.tb01666.x

[9] Craven, S.E., Stern, N.J., Line, E., Bailey, J.S., Cox, A. and Fedorka-Cray, P. (2000) Determination of the Incidence of

Salmonella spp. *Campylobacter jejuni* and *Clostridium perfringens* in Wild Birds near Broiler Chicken Houses by Sampling Intestinal Droppings. *Avian Diseases*, **44**, 715-720. http://dx.doi.org/10.2307/1593118

[10] Ahmed, A.M., Motoi, Y., Sato, M., Maruyama, A., Watanebe, H., Fukumotom, Y. and Shimamoto, T. (2007) Zoo Animals Reservoir of Gram-Negative Bacteria Harboring Integrons and Antibiotics Resistance Genes. *Applied and Environmental Microbiology*, **73**, 6686-6690. http://dx.doi.org/10.1128/AEM.01054-07

[11] Cheesbrough, M. (2005) District Laboratory Practice for Tropical Countries, Part 2. Cambridge University Press, Cambridge, 426 p. http://dx.doi.org/10.1017/CBO9780511581304

[12] Clinical and Laboratory Standards Institute (2007) Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI Document M100-S17. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

[13] Solomon, E.B., Yaron, S. and Matthews, K.R. (2002) Transmissino of *Escherichia coli* 0157:H7 from Contaminated Manure and Irrigation Water to Lettuce Plant Tissue and Its Subsequent Internalization. *Applied and Environmental Microbiology*, **68**, 397-400. http://dx.doi.org/10.1128/AEM.68.1.397-400.2002

[14] Beuchat, L.R. (1999) Survival of Enterohemorrhagic *Escherichia coli* O157:H7 in Bovine Feces Applied to Lettuce and the Effectiveness of Chlorinated Water as a Disinfectant. *Journal of Food Protection*, **62**, 845-849.

[15] Dahiru, M., Uraih, N., Enabulele, S.A. and Kawa, A.H. (2008) Prevalence of *E. coli* 0157:H7 in Some Vegetables in Kano City, Nigeria. *BEST*, **5**, 221-224.

[16] Uzeh, R.E. and Adepoju, A. (2013) Incidence and Survival of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on Salad Vegetables. *International Food Research Journal*, **20**, 1921-1925.

[17] Codex Alimentarius Commission (2003) Risk Profile for Enterohemorrhagic *E. coli*, Including the Identification of Commodities of Concern, Including Sprouts, Ground Beef and Pork. Codex Alinorm 03/13A: Report of Codex Committee for Food Hygiene 2003, Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Food Standards Programme, FAO, Rome, 60-64.

[18] Slama, T.G. (2008) Gram-Nagative Antibiotic Resistance: There Is a Price to Pay. *Critical Care*, **12**, 11-17. http://dx.doi.org/10.1186/cc6820

[19] Wang, S.H., Sheng, W.H., Chang, Y.Y., Wang, L.H., Lin, H.C., Chen, M.L., Pan, H.J., Ko, W.J., Chang, S.C. and Lin, F.Y. (2003) Healthcare-Associated Outbreak due to Pan-Drug Resistant *Acinetobacter baumannii* in a Surgical Intensive Care Unit. *Journal of Hospital Infection*, **53**, 97-102. http://dx.doi.org/10.1053/jhin.2002.1348

[20] Bonomo, R.A. and Szabo, D. (2006) Mechanisms of Multidrug Resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa*. *Clinical Infectious Diseases*, **43**, S49-S56. http://dx.doi.org/10.1086/504477