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Research Article

Antimicrobial susceptibility profile and ESBL-production of *Klebsiella* species isolated from duck cloaca.

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Abstract: Animals are known reservoirs of enteric bacteria and recent reports about the isolation of antimicrobial resistant bacteria from food animals have raised concern about the potential for food borne and zoonotic transmission. This study investigated the antimicrobial susceptibility pattern *Klebsiella* species isolated from duck cloaca from Igoli, Ogoja, Cross River State Nigeria. A total of 60 cloacal swab samples (2 from each) were asceptically collected from the cloaca of 30 duck from Igoli, Ogoja, Cross River State, Nigeria and immediately transported to Applied Microbiology Laboratory, Ebonyi State University, Abakaliki where they were analyzed by standard procedures. Isolates were properly identified as *Klebsiella* species using cultural/morphological characteristics, Gram reaction, biochemical characteristics and motility test and further subjected to Antimicrobial susceptibility Testing using the Kirby Bauer disk diffusion technique; their ESBL-production ability was as well tested. The results revealed a total of 14 isolates of *Klebsiella* species out of which 6(43%) were ESBL-producers whereas 8(57%) did not produce ESBL. Out of the 14 isolates, 12(85.7%) were resistant to Ceftazidime whereas 64.3% were susceptible to each of Aztreonam and Cefotaxime followed by Cefuroxime (57.1%). This study showed that *Klebsiella,* constituting members of pathogenic bacteria are present in food animals such as duck. This organism is not only present in high percentage (46.7%) but also resistant to conventional antibiotics as well as expresses the presence of ESBL. Therefore, as a result of high public threat revealed by this result, a good personal and environmental hygiene is hereby recommended and the indiscriminate use of conventional antibiotics in animal production should be prohibited.
Keywords: Antibiotics, *Klebsiella,* Ducks, Cloaca, Ogoja, zoonosis, ESBL, Resistance.

INTRODUCTION

The genus *Klebsiella* is made up of a group of non-motile, gram-negative, facultative aerobic, rod-like bacteria that ferment lactose [1]. They belong to the family of Enterobactericeae and forms a part of the aerobic bacterial flora of the intestine [2-3]. Based on hybridization studies Klebsiella is differentianed into eight different species and the first five species is clinically important[4]. As they are implicated in infectious diseases of the urinary tract, pelvis, tissues, they also also cause bacteremia, septicemia, endocardtis and pyogenic infections[5-6].

Cephalosporins and fluoroquinolones, aminoglycosides are known to be effective in treating infections caused by *Klebsiella spp*[7], regardless of the fact that Klebsiella spp are naturally resistant to ampicillin and carbenicilllim[8-9]. However recent studies have shown that resistance to multiple antibiotics have developed in this pathogen[8].

The emergence of antibiotics resistant bacteria is an issue that demands urgent attention due to the great threat it poses to the world's public health systems. This alarming development is an outcome of the unchecked indiscriminate use of antimicrobial agent; the self prescription of drugs without proper sensitivity test and overdosing, the uncontrolled use of antibiotics as prophylactics, growth promoters in animals[11]. Poor implementation of standard procedures in pharmaceutical process also contributes to this development [12-13]. Also, the microbial acquisition of the R-plasmids provides drugs resistance to cephalosporins and aminoglycoside in alarming frequencies [2].

Extended Specrum Beta-lactamase (ESBL) producing klebsiella spp have been of great concern in recent times. As it is on steady increase over the past few years, posing serious threats especially with regards to nosocomial infections. Also, antimicrobial co-rseistance to antimicrobial agents like quinolones and aminoglycosides antibiotics exists in ESBL-producing *Klebsiella spp.* As a result, both morbidity and mortality increases when infection is caused by these multi-drug resistant organisms [3].

Ducks are a source of meat, eggs and down-feathers (for making bedding and warm jackets). Duck meat and duck eggs are good dietary sources of high quality protein, energy and several vitamins and minerals [14]. However, they are potential carriers of resistant pathogenic enteric bacteria, which are capable of transmitting animal diseases to humans as a result of the various interactions with them as well as their environment [15]. The mode of breeding of ducks is a peculiar feature of ducks that could greatly enhance growth of microbial population as well as its dissemination to their immediate environment. As, these birds are usually found in clusters, as they aggregate around feeding areas and since they are aquatic , their habitat(streams, ponds, rivers) encourage microbial growth due to its high water activity and its vulnerability to contamination especially fecal contamination[16]. Also to note is the high level of mobility of ducks which greatly potentiates the cross contamination of microorganism to other animals as well as the environment especially through its droppings[17]. Microbiologically, it has been proven that the incessant and indiscriminate use of antibiotics in poultry production have led to the emergence of selected resistant strains of bacteria that form colonies in the intestines of these birds [18]. These bacteria are excreted leading to the contamination of the environment, meat and other products meant for human consumption [19-21]. Thus, these birds reservoirs of resistant pathogenic bacteria at the expense of public health . Much more alarming, is the clinical reports that exist in increasing proportions which reveals that these resistant bacteria resident in the entrails of these birds are not just transmissible to man but also cause infections which becomes difficult and recalcitrant during treatment, thus having serious clinical implications as well as economic implications[19, 22].

In addition, the selective pressure and use of sub-inhibitory concentration of antibiotics among human population have greeted this condition with severe clinical prospects. As most of these bacteria become resistant to antibiotics, multiple antibiotics and even start producing ESBL when they acquire extra chromosomal genetic components through mutation or any other means. These genetic components include resistance (R)-plasmids, transposons and integrons which carry the genes that encodes for resistance [18]. One great concern is the spread of positive ESBL strains in hospitals which may lead to outbreaks or to endemic occurrences [23]. Also, the limited therapeutic choices in treating infections caused by ESBL-producing strains is equally a threat that needs to be curbed as high mortality is more associated with ESBL-producing microbes than other microorganism who do otherwise[8][24]. This investigation was undertaken to give insight about the antimicrobial susceptibility pattern of *Klebsiella spp* isolated from duck cloaca and also determine the prevalence of ESBL-producing strains among the isolates. The result revealed a high presence of resistant *Klebsiella* species among which were ESBL-producers.

MATERIALS AND METHODS

Sampling

Cloacal swabs were aseptically obtained using standard procedures in Applied Microbiology Laboratory, Ebonyi State University, Abakaliki, from 30 different species of ducks transported from Igoli, Cross River, Nigeria. Cloacal swabs were obtained by inserting a sterile swab stick into the cloaca and rotating the tip against the mucosa gently. The swabs were well labeled and kept under ambient temperatures till inoculation.

Isolation and Identification of *Klebsiella* spp

The cloacal swabs of the ducks were streaked on the Eosin Methylene Blue (EMB) and Mac-Conkey agar and incubated for 24 hours at 37^{o}C. After 24hrs, preliminary examination of the agar plates was made basis of colonial characteristics. Further identification procedures were also carried out, which includes:

Microscopic Examination:

This was used to determine the motility of the isolates using the Wet mount method[26]and also their gram reaction.

Biochemical identification:

Catalase test, Indole test, Citrate Utilization test, Oxidase test, Voges Proskauer(VP) test, Methyl red test were carried out for every isolate to determine the biochemical characteristics.

Antibiotic Susceptibility testing

The antibiotic susceptibility test was performed using the Kirby-Bauer's disc diffusion method on Mueller Hinton Agar. The bacterial culture was prepared by inoculating the colonies into sterile distilled water to give the inoculums turbidity equivalent to a 0.5 McFarland turbidity standard and swabbed evenly onto agar plates and incubated for 37_{o}c for 24hrs. Zones of inhibition were measured and interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards [26].The isolates were used against these antibiotics Cefurotriaxone (CRO), Aztreonam(AZT), Cefuroxime (CXM), Cefotaxime (CAZ).

Screening Isolates for ESBL production

ESBL production was tested by using ceftazidime (30 mcg) plus clavulanic acid (30/10 mcg) discs on Mueller-Hinton agar. Organisms are considered producers if there was a ≥5mm increase in zone diameter around ceftazidimne/ clavulanic acid disc compared to zone around ceftazidime alone. ESBL production was tested in parallel with the antibiotic testing on a separate Mueller Hinton Agar plate in line with theNCCLCS guidelines.

RESULTS

A total of 30 cloacal swabs were taken from thirty different ducks. A total of 14 *Klebsiella spp*isolates (D_1, D_2, D_3....D_{14}) were recovered from the samples used for the study (Table 1). Also, results of colonial/morphological characteristics of the *Klebsiella*spp isolates indicated that all the isolates from the samples produced pink and mucoid colonies on MacConkey & Eosine Methylene Blue (EMB) agar. Also they were all gram-negative rods which were in chains with regards to their arrangement (Table 1). Table 2 reveals the results the biochemical tests carried out for the identification of the *Klebsiella*spp isolates. This result showed that the 14 *Klebsiella*spp isolates were all positive to citrate, catalase, and voges-proskauer tests but gave negative reactions to indole, methyl red and oxidase tests.

Table-1: Morphological Characteristics of *Klebsiella* species on MacConkey and Eosine Methylene Blue (EMB) agar.

Sample Codes	Gram reaction	Motility	Colour On EMB	Colour On MacConkey	Suspected Organism
D_1	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_2	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_3	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_4	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_5	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_6	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_7	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_8	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_9	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_{10}	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_{11}	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_{12}	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_{13}	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp.
D_{14}	-ve	Non-motile	Pink & mucoid	Pink & mucoid	*Klebsiella* spp

Table-2: Biochemical Characteristics of *Klebsiella* species isolated from Duck cloaca.

Isolates	Biochemical Characteristics						
	Citrate	Indole	Catalase	Voges-Proskauer	Methyl Red	Oxidase test	Suspected Organism
D_1	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_2	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_3	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_4	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_5	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_6	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_7	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_8	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_9	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_{10}	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_{11}	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_{12}	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_{13}	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp.
D_{14}	+ve	-ve	+ve	+ve	-ve	-ve	*Klebsiella* spp

The antimicrobial susceptibility profile of the *Klebsiella*spp isolates is shown in Table 3 based on the microbiological standards for antimicrobial testing shown , the profile reveals that isolates D_5, D_7, D_{10}, D_{12} were highly susceptibility to aztreonam with diameter of zones of inhibition of 35mm, 34mm, 34mm and 33mm respectively. While ceftzidime had no inhibitory effect on isolates D_3, D_4, D_5, D_6, D_7, D_8, D_{10}, D_{12}, D_{13}, D_{14}, as they were highly resistant to the antibiotic. Also, revealed in Table 3 is that 4(33.3%) isolates (D_3, D_5,

D_9, D_{11}) were resistant to two or more antibiotics, some were susceptible while others were intermediately susceptible to two or more antibiotics when compared to the standard.

Table-3: Antimicrobial Susceptibility profile of *Klebsiella* species isolated from duck cloaca.

Isolates	Antibiotics				
	CRO	**ATM**	**CXM**	**CAZ**	**CTX**
K_1	**25**	**15**	**25**	**16**	**25**
K_2	**23**	**30**	**21**	**21**	**30**
K_3	**NI**	**12**	**NI**	**NI**	**12**
K_4	**20**	**32**	**23**	**NI**	**29**
K_5	**30**	**35**	**NI**	**NI**	**32**
K_6	**19**	**30**	**19**	**NI**	**21**
K_7	**25**	**34**	**25**	**NI**	**33**
K_8	**19**	**27**	**24**	**NI**	**30**
K_9	**NI**	**6**	**10**	**10**	**15**
K_{10}	**23**	**34**	**30**	**NI**	**33**
K_{11}	**NI**	**8**	**NI**	**NI**	**13**
K_{12}	**20**	**33**	**24**	**NI**	**29**
K_{13}	**NI**	**14**	**30**	**NI**	**35**
K_{14}	**24**	**31**	**25**	**NI**	**32**

Key: NI =No inhibition**,** CRO =Cefurotriaxone**,** ATM =Aztreonam**,** CXM =Cefuroxime**,** CTX =Cefotaxime**,** CAZ=Ceftazidime

Table-4: Percentage (%) Susceptibility Pattern of *Klebsiella* species from duck cloaca.

	Antibiotics				
	CRO	**ATM**	**CXM**	**CAZ**	**CTX**
% Susceptibility	42.9	64.3	57.1	7.1	64.3
% Resistance	28.6	35.7	28.6	85.8	35.7
% Intermediate	28.6	0	14.3	7.1	0

Key: CRO =Cefurotriaxone, ATM =Aztreonam, CXM =Cefuroxime, CTX =Cefotaxime, CAZ =Ceftazidime

Results of this study reveals that the isolates showed a high degree of susceptibility to aztrenoam (64.3%) and Cefotaxime (64.3%) followed by cefuroxime (57.1%), ceforatrixone (42.9%) and cefurotriaxone (7.1%). 85.7% of the isolates were highly resistant to ceftazidime while 35.7% was resistant to aztronam, 28.6% was resistant to cefuratriaxone and cefuroxime. While 28.6% and 14.3% of the isolates were intermediately susceptible to cefurotriaxone and cefuroxime respectively (Table 4, Figure-1).Out of the 14 isolates of *Klebsiella* spp., 6(43%) were identified to be ESBL-producers while 8(57%) were not ESBL-producers; details this prevalence of ESBL-producers among the isolates is shown in Table 5 and Figure-2.

Table 5. Prevalence of ESBL-producers among *Klebsiella* species isolated from duck cloaca.

Isolate	ESBL production
K_1	**+ve**
K_2	**-ve**
K_3	**-ve**
K_4	**+ve**
K_5	**-ve**
K_6	**-ve**
K_7	**+ve**
K_8	**-ve**
K_9	**-ve**
K_{10}	**+ve**
K_{11}	**-ve**
K_{12}	**-ve**
K_{13}	**+ve**
K_{14}	**+ve**

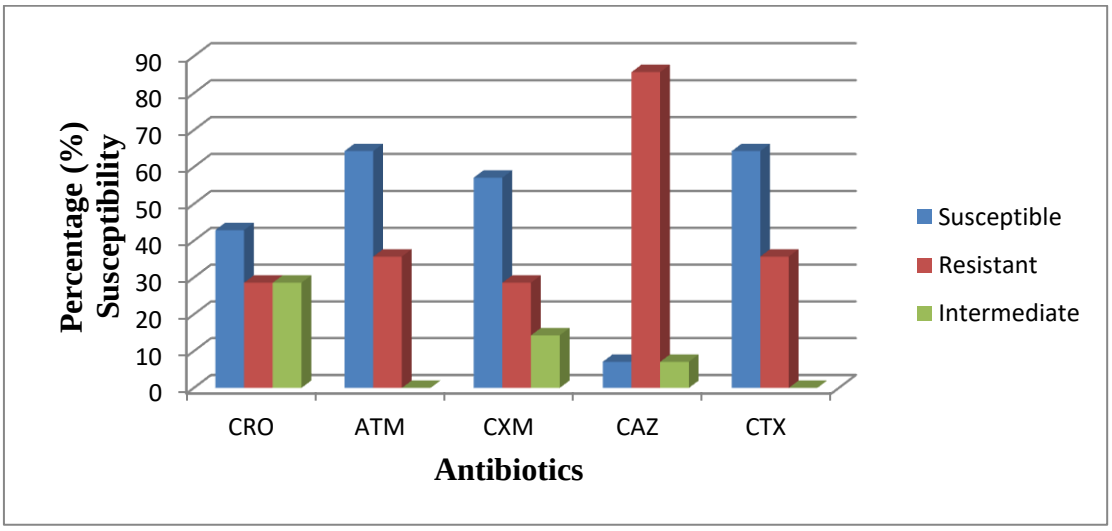

Fig-1: Percentage (%) Susceptibility Pattern of *Klebsiella* species from duck cloaca.
(CRO =Cefurotriaxone, ATM =Aztreonam, CXM =Cefuroxime, CTX =Cefotaxime, CAZ =Ceftazidime)

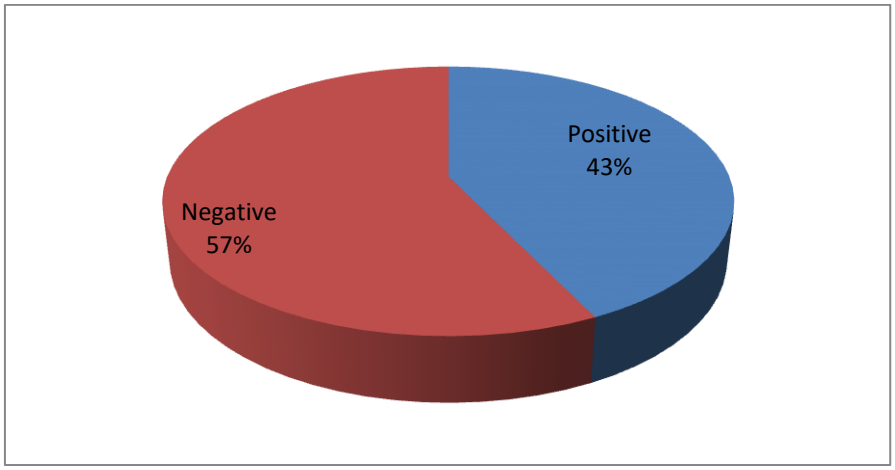

Fig-2:Percentage (%) Prevalence of ESBL-production among *Klebsiella* species isolated from Duck cloaca.

DISCUSSION

Klebsiella like every other microorganism is ubiquitous in nature, however its common habitat is usually surface water, sewage, soil and plant surfaces [25]. Ducks are mostly aquatic birds, mostly smaller than the swan and geese and may be found in both fresh water and sea water [14].Also, Ducks are potential carriers of pathogenic bacteria which are capable of transmitting zoonotic diseases to human as a result of the various interactions man had with them since they are domesticated animals.

The findings from this study showed that 14 isolates of *Klebsiella*spp were obtained from the samples under study .The presence of *Klebsiella*spp in the cloaca swabs could be as a result of faecal contamination of the habitat (usually water) or environment where the ducks bred, as *Klebsiella*spp are equally indicators of fecal contamination. Also,it can also be deduced from this report that *Klebsiella* is a member of enterobacteriaceae family and should be found in the gastrointestinal tract of animals [17].

Table 4.5 showed that ceftazidime was the least effective antimicrobial agent against the isolates, as 12(85.7%) out of 14 isolates were resistant to the antimicrobial agent.This report is in consonance with reports of Gundogan and Avci [25] in Turkey and Akujiobi[2] in Abakaliki, Nigeria which recorded resistance of *Klebsiella spp* isolates to this third generation cephalosporin. This pattern of resistance suggests that this antibiotics have been exposed to selective pressure due to the wide spread, indiscriminate use of ceftazidime in the environment or in enhancing the feeds given to the ducks. Resistance of the isolates could also be as a result of ESBL which mediates resistance in β-lactams agents like cefotaxime, ceftriazone, ceftazidime[28].

Also, these isolates showed susceptibility to aztreonam (64.3%), cefotaxime (64.3%), however this contradicts the reports of Gundogan and Avuzi [25] as they reported resistance o*f Klebsiella* spp isolates from a study in Turkey. This could be as a result in geographical location as well as the differences in the availability of these antimicrobial agents for

consumption and use, as antibiotic susceptibility pattern may change from time to time and place to place [3]. The differences in the origins of the isolates could also be a contributing factor.

From table 4.4, it is seen that 4(33.3%) of the isolates were resistant to two more antimicrobial agents. However, this is not in tandem with the findings of Gundogan and Avzi [25] and that OF Ullah et al[8] in Pakistan . This contrast could be traced to the differences in the nature, availability and the distribution of the antimicrobial agents used for the study. However , multi-drug resistance may be due to the presence of plasmids which carry several resistance genes and are capable of transferring it from one bacterium to one another[8](ram and gupta ,2000). Also studies have shown that multi-resistance in klebsiella spp can be linked to the presence of integrons [29-30].

The prevalence of ESBL-producers among the *Klebsiella* spp isolates is 43% from the results of this study. These findings suggest the presence of plasmids in some isolates as ESBL-encoding genes are often carried on plasmids which can easily be transferred between isolates. This makes it easy for these isolates to bear additional resistance determinants in their plasmids for other classes of antimicrobial agents contributing to their ability to exhibit multi-drug resistance [31].

CONCLUSION

From the study it is clear that ducks are reservoirs of resistant bacteria which have lots of potential to contaminate the environment. These domestic birds are also important sources of dissemination of resistant strains of the *Klebsiella*spp as well as strains with the ESBL-encoding genes to humans and the environment. Against this development, it becomes imperative that further studies be carried out to investigate the emergence of resistant, ESBL-producing *Klebsiella*spp together with the predisposing factors .Furthermore, continuous surveillance for ESBL-producing *Klebsiella*spp should be instituted at various levels (domestic birds, humans, and environment) including factors that aid their distribution in communities.

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