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Microbial Etiology of Duck Mortality in Odisha, India

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A B S T R A C T

In the present study 128 samples collected from 66 ducks of various breeds like Batak(28), Moti(34) and Khaki Campbell (6) out of which 112 number of samples from 52 dead birds and 16 number of samples from live birds. blood, liver, swabs from femur bone marrow and also swabs from foot lesions were collected consists of 128 samples in which blood sample (12), swabs from foot lesions(26) of live birds and liver(22), lungs(30) and swab from bone marrow (38) of dead birds. All the samples were processed for routine microbial isolation and identification. *Pasteurella* species was found to be the most predominant isolate (16%) followed by Pseudomonas species was found to be (11.2%) and *E.coli* (55%) where as fungal isolates like *Aspergillus* species was found to be (18%). *Pasteurella* species were cultivated in 5% sheep blood agar and McConkey's agar whereas *Aspergillus* species in SDA by routine inoculation procedure. The ducks were ranging from 2 to 16 weeks of age mostly from unorganised sectors of Odisha. Ducklings were found to be more prone to Pseudomonas and *Aspergillus* species in comparison to adult ones on antibiotic sensitivity test.it was revealed that tetracycline, doxycycline, oxytetracycline, found to be least sensitive in disk diffusion method. Whereas gentamicin, and ciprofloxacin were found to be highly sensitive against *E.coli* and gentamicin, amikacin, tobramycin were found to be highly sensitive against *Pseudomonas* species. Most of the gram negative bacteria isolated in the present study like *Pasteurella* species, *Pseudomonas* species and *E. coli* species were found to be resistant to tetracycline, amphotericin-B. griseofulvin were found to be highly sensitive for *Aspergillus* species isolated in the present study.

Introduction

Mostly ducks are reared in coastal districts of odisha like kendrapada, cuttack, puri and paradeep and rural parts of Odisha. Mainly indigenous ducks are reared for where there is source of water in the form of ponds. These indigenous water fowls are of two types one in Batak and other in Moti (Muscovy). According to 2003 data the total numbers of

ducks present in Odisha was 6,10,000. Commercial rearing of khaki Campbell ducks are practised in coastal areas of Odisha. So in order to treat the water fowls expediently a vivid knowledge regarding the common causative agents of the infectious diseases and its antibiotic sensitivity profile is of paramount importance.

Materials and Methods

Sample collection

In the present study a total number of 128 dead and live duck samples were collected from kendrapada, Puri, Paradeep and ADRI (Animal Disease Research Institute) Cuttack during the period of December, 2018 to May, 2019. Different organs like liver, lungs, swab sample from bone marrow of dead birds and blood sample, swabs from foot lesions were collected from live birds and processed for routine microbial isolation (Fig. 1).

Microbial analysis

The samples were inoculated in standard broth like Brain heart infusion broth (BHI) and incubated at 37°c for 24 hrs. Various selective media like Pseudomonas isolation agar, McConkey lactose agar and blood agar were used for isolation of different bacteria. Identification of the organism was done with reference to colony morphology (size, colour and nature), pigment production along with various biochemical test.

Mycological analysis

For fungal isolation samples were inoculated into Sabouraud dextrose agar (SDA) and Potato dextrose agar(PDA) and incubated at 37°c ngfor 3-4 days. Identification of the fungus has carried out by lactophenol cotton blue staining along with observing colour, hyphae and nature of colony.

Antibiogram study

In-vitro antimicrobial study was carried out by using Muller Hinton agar M/S Hi-Media Laboratories Ltd. Mumbai for bacterial isolates and Muller Hinton agar with 2% glucose and 0.5% mcg/ml methylene blue was used for fungal isolates (Bauer *et al.*, 1966). The antibiotic discs like ciprofloxacin (CIP-30mcg), ampicillin (AMP-10mcg), tetracycline (TE-1mcg), doxycycline (DO-30mcg), erythromycin (E-10mcg), chloramphenicol (C-20mcg), cotrimoxazole (COT-25mcg), amoxyclav (AMC-30mcg), gentamicin (GEN-10mcg), and Amikacin (AK-30mcg) were used for bacterial isolates. Griseofulvin (GS-), Amphotericin-B(AP-20mcg), Tobrmycin (TOB-30mcg) ketoconazole (KT-50mcg) and fluconazole (FLC-10 mcg) were used for fungal isolates. All the disc were obtained from Hi-Media, Mumbai, India. The results were recorded according to criteria set by clinical and laboratory standards institute (CLSI) (Wayne, 2009).

Results and Discussion

In the present study, 90 bacterial isolates and 37 number of fungal isolates were obtained by routine microbial isolation. *E.coli* (70/127) was found as the most predominant bacterial isolates (Adziety *et al.*, 2011) followed by *Pasteurella species* (20/127). Similarly *Aspergillus species* (23/127) was the predominant fungal isolates followed by *Pseudomonas species* (14/127) which shows 18% of the fungal infection out of total sample collected. This result is similar to that of study of (Samuelson *et al.*, 1984) Pink colour colony was produced by *E.coli* (n=70) on McConkey lactose agar whereas *Pasteurella species* (n=20) produces small, glistening and dew drop like colony on blood agar plate this result is in agreement with the findings of (Bhattacharya, 2005). *Aspergillus*

species produces characteristic wooly and green colonies where as *Pseudomonas species* flurescin pigment (greenish colour) on MHA plates. Club shaped vesicle with unseriate conidia was identified on lactophenol cotton blue staining of *Aspergillus species.* on analysis of antibiogram susceptibility pattern produced by various antibiotics and antifungals, it was found that *E.coli* was highly sensitive to and gentamicin and cipro loxacin. *Pasteurella* species isolates were sensitive to amoxyclav, chloramphenicol, gentamicin and co-trimoxazole. This study is in agreement with (Bauer *et al*., 1966) and (Bhattacharya A. 2005). Pseudomonas species isolates were sensitive to gentamicin, amikacin and tobramicin and *Aspergillus* species isolates were highly sensitive to griseofulvin (Fig. 2–5).

Table.1 The distribution of samples collected from various breeds of Ducks (n=66)

Si no.	Breed	age	Dead birds			Live birds	
			Liver	Lungs	Swabs of femur bone	Swabs of foot lesions	Blood
1.	Indigenous Batak (n=30)	0-4 week (n=20)	5	10	9	-	-
		4-16 week (n=10)	3	4	7	8	5
2.	Indigenous Moti(Muscovy) (n=25)	0-4 week (n=15)	4	5	7	-	-
		4-16 week (n=10)	3	4	6	11	3
3.	Khaki Campbell (n=11)	0-4 week (n=7)	4	4	5	-	-
		4-16 week (n=4)	3	3	4	7	4

Table.2 Prevalence and isolation of different microbial isolates from duck samples

Name of the Bacterial isolates	No. of isolation	Isolation (%)
E.coli	70	55
P.multocida	20	16
Aspergillus species	23	18
Pseudomonas species	14	11.2

Table.3 Comparative study of antimicrobial sensitivity test for bacterial isolates

Si no.	Bacterial isolates	CIP	AMP	TE	DO	E	C	COT	AMC	GEN	TOB	AK	GS	AP	KT	FLC
1.	***E.coli***	21	13	11	12	14	13	-	-	15	-	-	-	-	-	-
2.	***P.multocida***	21	-	-	-	-	18	16	18	15	-	15	-	-	-	-
3.	***Pseudomonas species***	16	-	-	-	-	12	-	-	15	15	17	-	-	-	-
4.	***Aspergillus species***	-	-	-	-	-	-	-	-	-	-	-	21	17	20	20

CIP-Ciprofloxacin, AMP-Ampicillin, TE-Tetracycline, DO-Doxycycline, E-Erythromycin, C-Chloramphenicol, COT-Co-trimoxazole, O-Oxytetracycline, AMC-Amoxyclav, GEN-Gentamicin, AK-Amikacin, GS-Griseofulvin, AP-Amphotericin-B, KT-Ketoconazole, FLC-Fluconazole
*Not all microbials are tested against all microbial isolates

Figure.1 Khaki Campbell duck

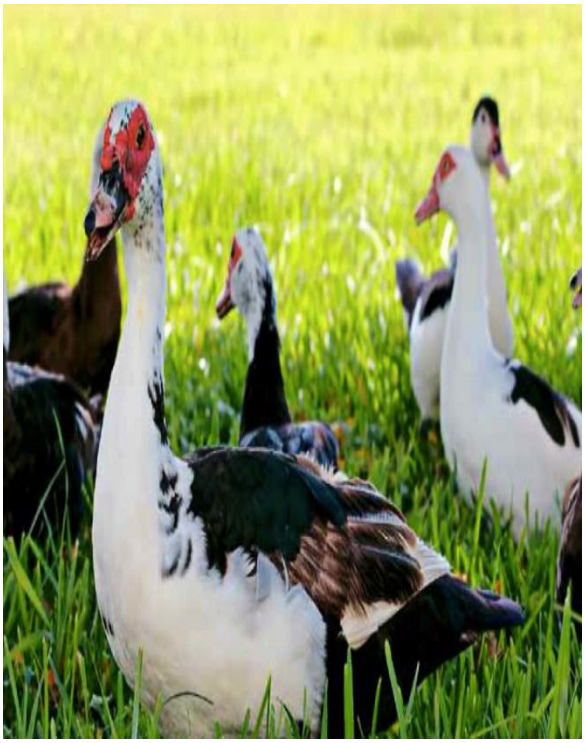

Figure.1(b) Muscovy(Moti) duck

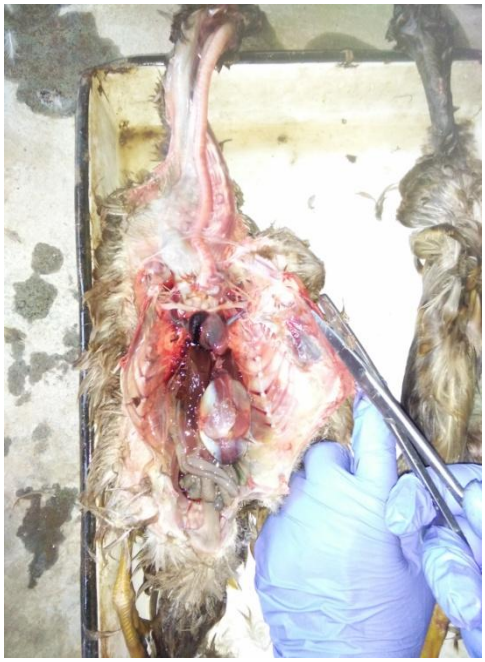

Figure.1(c) PM of dead duckling

Figure.1(d) dead khaki Campbell duckling

Fig.2 Collection of sample for khaki Campbell and white pekin breed.

Fig.3 *E. coli* culture on MLA plate

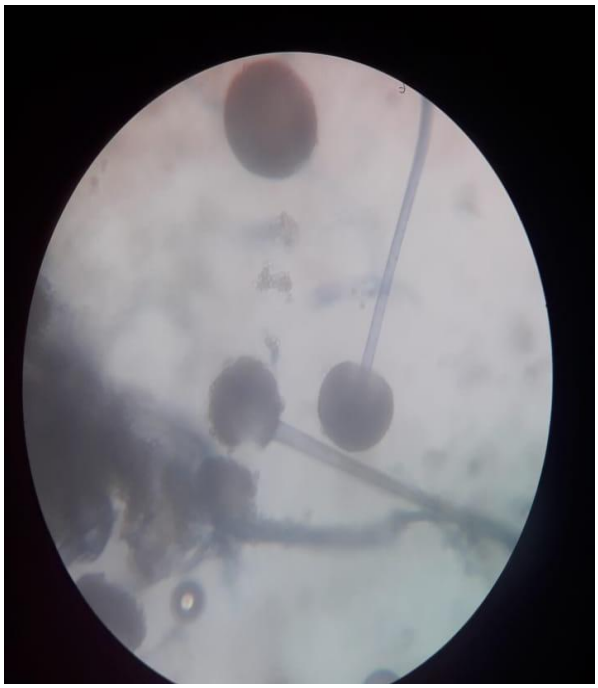

Fig.4 Lactophenol cotton blue staining of *Aspergillus* species

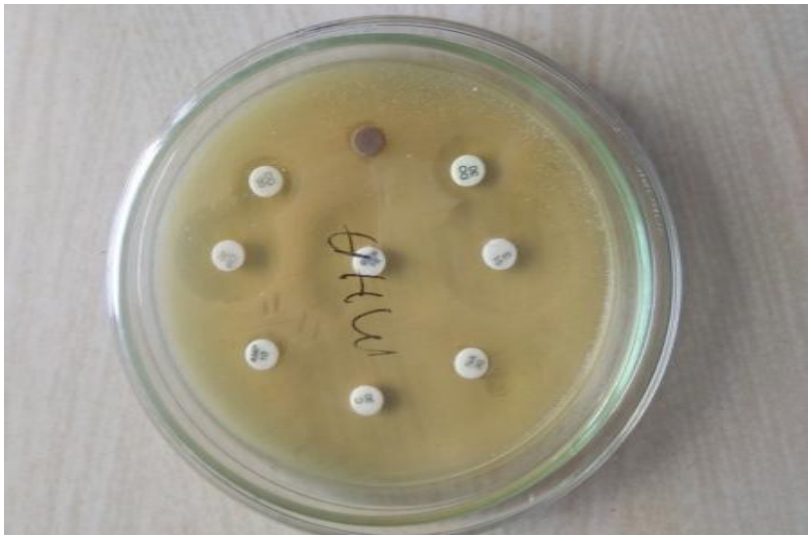

Fig.5 Antibiotic sensitivity plate for *E. coli*

In case of *E.coli* antibiotics like ciprofloxacin, ampicillin, tetracycline, doxycycline, erythromycin, chloramphenicol and gentamicin showed inhibitory zone of 21, 13, 11, 12, 14, 13, and 15 mm respectively. In case of *Pasteurella species* ciprofloxacin, chloramphenicol, cotrimoxazole, amoxyclav, gentamicin and amikacin showed inhibitory zone of 21, 18, 16, 18, 15 and 15 mm respectively. In case of *Pseudomonas species* gentamicin, tobramicin, amikacin, ciprofloxacin, and chloramphenicol showed inhibitory zone of 15, 15, 17, 16, and 12 mm respectively. Similarly, in case of *Aspergillus species* griseofulvin, amphotericin-B, fluconazole and ketoconazole showed inhibitory zone of 21, 17, 20 and 20mm, respectively. Inhibitory zone of different microbial can be found in table no.3.

Therefore it is concluded, on the basis of present observation the most prevalent micro organism in indigenous ducks of Odisha was *Pseudomonas* species followed by *Pasteurella* species, *E.coli* whereas fungal agent like Aspergillosis was more common in ducklings (0 to 4 weeks of age). Further study in more detail in qualitative microbial load is required to combat the disease prevalence in densely populated indrustrialized duck production.

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