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Case Study

INCIDENCE OF COLIBACILLOSIS IN KINGFISHER BIRD

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ABSTRACT
A common Kingfisher bird (*Alcedo atthis*) was presented with clinical signs of respiratory distress, depression, weakness, yellowish diarrhoea, vent pasted with faeces and died suddenly. After death, the post-mortem findings lay the first foundation of *E. coli* infection which was later confirmed by bacteriological and biochemical tests. The antibiotic sensitivity test revealed results that were specific for the bird under study.

KEY WORDS: Incidence, Colibacillosis, Kingfisher bird.

INTRODUCTION

Escherichia coli is a rod-shaped, Gram-negative, facultative anaerobic bacterium that belongs to the *Enterobacteriaceae* family. *E. coli* typically colonizes the intestinal tract of humans (Drasar and Hill, 1974), mammals and birds (Sokja, 1965). *E. coli* strains are usually found confined to the intestinal lumen, however in imunosuppressed hosts or when the gastrointestinal barriers are violated, these bacteria may cause infectious diseases (Nataro and Kaper, 1998). Besides that, there are *E. coli* strains originated from adapted pathogenic clones that cause diseases among healthy animals (Nataro and Kaper, 1998). Pathogenic *E. coli* are associated with intestinal (Nataro and Kaper 1998) and extraintestinal human infections, like cystitis and pyelonephritis (Pechere, 1985), septicemia (Orskov and Orskov, 1985) and meningitis in neonatal infants (Overall Jr, 1970). Among other animals, pathogenic *E. coli* strains are also able to cause extraintestinal infections such as urinary infection (Wooley and Blue, 1976, Peeters, 1994) and pyometra (Bjurstrom, 1993) in dogs, and respiratory diseases in birds (Kaper *et al*., 2004). Currently, *E. coli* is considered to be the most significant example of gram-negative bacterium related with diverse diseases because of the different pathogenicity mechanisms and diseases that it is able to cause. Avian pathogenic *E. coli* strains are known as APEC (Dho-Moulin and Fairbrother, 1999) and are associated with diverse diseases, mainly extraintestinal, being responsible for great losses in the avian industry (Gross, 1994). Colibacillosis was rporetd by many researchers in different countries(omer *et al*.,2008,Vandekrchoveet al.,2004; Yang *et al*, 2004,Saenz *et al*.,2003, White *et a*l.,2000) . therefor the diseas is considred one of the principal causes of mortality and morbidity in birds. Inflammation of the oviduct causes in decreased egg production and sporadic mortality in laying chickens and breeders, salpingitis that occurs when *E. coli* ascends the oviduct from the cloaca and extension into the body cavity through the compromised oviduct wall that leads to concurrent peritonitis (Barnes *et al*., 2003).

History and Clinical signs

A common Kingfisher was presented with clinical signs of respiratory distress, depression, weakness, yellowish diarrhoea, vent pasted with faeces and died suddenly.

Post-mortem findings

After death the post-mortem examination was conducted. Necropsy revealed characteristic lesions in the liver, intestine, and gall bladder. The liver was enlarged, pale, friable and necrotic (Fig. 1). The gall bladder was distended with bile and haemorrhages were seen in the intestine (Fig. 2) (Omer *et al*., 2010). Also pericarditis was recorded as one of the prominent lesions (Fig. 1).

Isolation and identification of bacteria

After conducting necropsy, a small part of affected intestine was processed for bacteriological examination. The collected Sample was inoculated on Nutrient Broth and incubated at 37ºC for 24 hours and then transferred on selective media (Mac Conkey agar and Eosin methylene blue agar). The colonies were examined with naked eye for their cultural characteristics, morphological properties and any changes in the media. Gram-stained slides were obtained which were examined microscopically. The intestinal culture yielded round colonies with typical metallic sheen in Eosin methylene blue (EMB) agar (Fig. 4) (Horvath and Ropp, 1974). The colonies on MacConkey lactos agar appeared small, round and pink coloured (Fig. 3) (March and Ratnam, 1986). Gram stained smear showed uniform gram negative bacilli.

Biochemical tests of the isolate

The isolate was identified by using standard biochemical test kit (KB001 TM HiIMViC, India) (Fig. 5) including, indol, methyl red, voges praoskauers, citrate utilisation and carbohydrate fermentation test (glucose, Adonitol, arabinose, lactose, sorbitol, manitol, Rhamnose and sucrose). The isolate was biochemically identified as

E.coli using standard biochemical test kit (Omer *et al*., 2010).

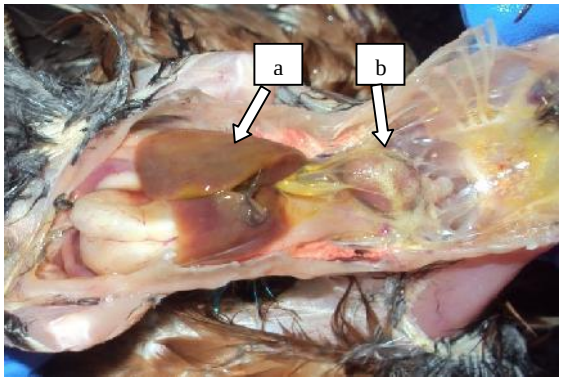


FIGURE 1: Figure showing lesions in a) liver and b) heart of a kingfisher bird affected with Colibacillosis

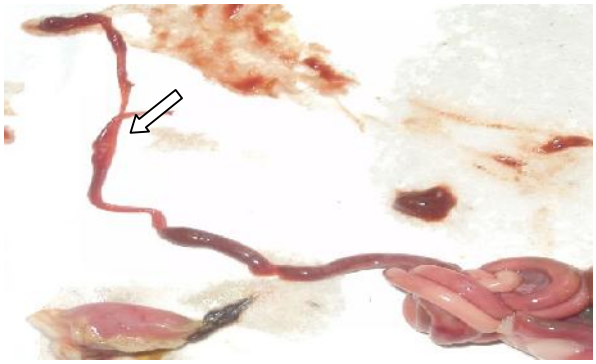


FIGURE 2: Figure showing haemmorrhagic lesions (arrow) in the intestine of a kingfisher bird affected with Colibacillosis

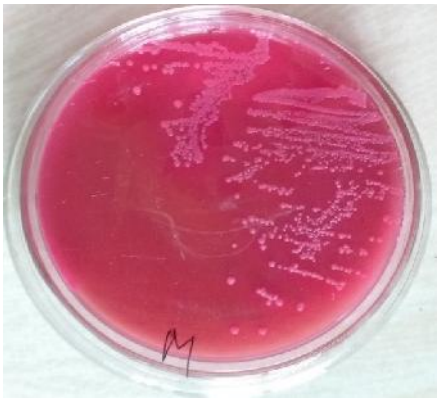
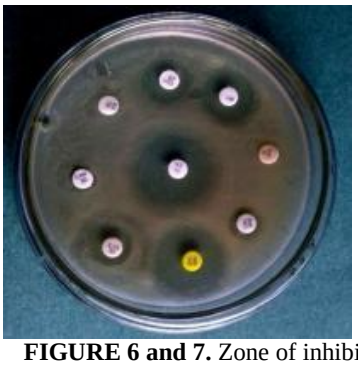


FIGURE 3: Figure showing pink colonies of E.coli on Mac conkey lactose Agar

FIGURE 4: Figure showing metallic sheen appearance of *E. coli* colonies on Eosin Methylene Blue Agar

FIGURE 5: KB001 TM HiIMViC, India Biochemical test Kit



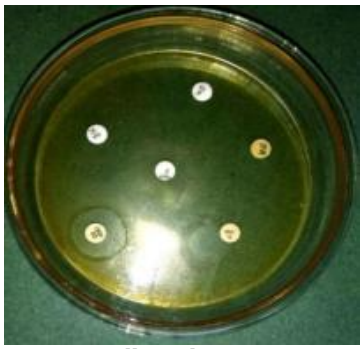


FIGURE 6 and 7. Zone of inhibition on Mueller Hilton Agar using Kirby Bauer disk diffusion method

Antimicrobial susceptibility test

According to the standard operational procedures, antimicrobial susceptibility tests were done against 14 antimicrobial drugs on Mueller-Hilton agar (Oxoid, Hampshire, England) using Kirby Bauer disk diffusion method (Fig. 6 and Fig. 7). The antimicrobial agents tested were: Tetracycline (30 µg), Nitrofurantoin (300 µg), Erythromycin (15µg), Chloramphenicol (30µg), Gentamicin (10µg), Co-Trimoxazole (25µg), Ceftriaxone (30µg), Norflaxocin (10µg), Amoxicillin- Clavulinic acid (20/10µg), Nalidixic acid (30µg), Ampicilin-cloxacilin (10µg), Cefuroxime (30µg), Ceftriaxone (30µg), Piperaciline-Tazobactam (100µg), Ampicilin (10µg). Resistance data were interpreted according to National Committee for Clinical laboratory Standards (NCCLS) (Table 1). The isolate was sensitive to Gentamicine, Norfloxacin, Nitrofurantoin, howeverit was intermediately sensitive to Chloramphenicol and Co-Trimoxazole. It was completely resistant to Tetracycline, Erytromycin, Amoxicilin-Clavulinic acid, Nalidixic Acid, Ampicilin-Cloxacilin, Cefuroxime, Ceftriaxone, Piperacilin-Tazobactam and Ampicilin.

TABLE 1: Antimicrobial sensitivity profile of E.coli isolate

Sl. No.	Name of the anti-microbials	Zone of inhibition (mm)	Sensitivity
1	Gentamicine	15	Senitive
2	Norfloxacin	22	Sensitive
3	Nitrofurantoin	19	Sensitive
4	Cotrimoxazole	13	Moderately sensitive
5	Chloramphenicol	17	Moderately sensitive
6	Tetracycline	0	Resistant
7	Erythromycin	0	Resistant
8	Amoxyclav	0	Resistant
9	Nalidixic acid	0	Resistant
10	Ampicilin+cloxaciin	7	Resistant
11	Cefuroxim	0	Resistant
12	Ceftriaxone	0	Resistant
13	Piperacilin+tazobactam	8	Resistant
14	Ampicilin	0	Resistant

CONCLUSION

It is apparent that APEC causes multi-factorial diseases, which involves host-pathogens interactions. The results of this study show high rates of antimicrobial resistance to Tetracycline, Erytromycin, Amoxycilin-Clavulinicacid, Nalidixic Acid, Ampicilin- Cloxacilin, Cefuroxime, Ceftriaxone, Piperacilin-Tazobactam and Ampicilin. Nitrofurantoin, Norfloxacin and Gentamicin are considered appropriate for empirical treatment of *E. coli*. Further, molecular and biological characterization linked to appropriated animal models is needed to better understand the APEC pathogenesis. The incidence of colibacillosis in common Kingfisher bird is successfully reported here.

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