ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Asian Journal of Animal Sciences 7 (2): 64-69, 2013
ISSN 1819-1878 / DOI: 10.3923/ajas.2013.64.69

Presence of Extended Spectrum β-lactamases Producing α Haemolytic *Escherichia coli* in Yellow-wattled Lapwing (*Vanellus malabaricus*)

[1]Amit Kumar, [2]Amit Kumar Verma, [1]Arvind Kumar Sharma and [3]Anu Rahal
[1]Department of Veterinary Microbiology and Immunology,
[2]Department of Veterinary Epidemiology and Preventive Medicine,
[3]Department of Veterinary Pharmacology and Toxicology, Uttar Pradesh Pandit Deen Dayal Upadhaya Pashu Chikitsa Vigyan Vishwavidhyalaya Evam GoAnusandhan Sansthan, Mathura-281001, Uttar Pradesh, India

Corresponding author: Arvind Kumar Sharma, Department of Veterinary Microbiology and Immunology, Uttar Pradesh Pandit Deen, Dayal Upadhaya Pashu Chikitsa Vigyan Vishwavidhyalaya, Evam GoAnusandhan Sansthan, Mathura-281001, Uttar Pradesh, India

ABSTRACT

Since last decade, there is increasing reports of presence of extended spectrum β-lactamases producing bacteria especially from enterobacteriacae family. The presence of Extended spectrum β-lactamases (ESBL) producing *E. coli* causes a serious public health threat to human beings. The present study reports the isolation of extended spectrum β-lactamases producing α hemolytic *Escherichia coli* in Yellow-wattled Lapwing (*Vanellus malabaricus*) chicks. *Escherichia coli* organisms were isolated from three chicks suffering from unusual clinical signs and died before rehabilitation in the month of August 2010. Further assessment of isolates revealed their ability to bind with Congo red dye on Tryptic soy agar media and α hemolytic nature on 5% chicken blood agar. As usual when drug sensitivity was performed it revealed their multi drug resistance pattern which on further examination with double disk method showed them to be extended spectrum β-lactamases producing *Escherichia coli*. The presence of enterohemorrhagic extended spectrum β-lactamases producing *Escherichia coli* in Yellow-wattled Lapwing is a matter of concern as it may be due to the transmission from human being as these Lapwings are residing in the close vicinity of university premises. Moreover, their nesting areas are also very near to the place where carcasses of dead animals were disposed off during that period of time. To the best or our knowledge, this appears to be the first report of pathogenic *E. coli* identified in Yellow-wattled Lapwing, implicating Yellow-wattled Lapwings as a new potential reservoir of these zoonotic pathogens.

Key words: Extended spectrum β-lactamases (ESBL), *Escherichia coli,* yellow-wattled lapwing, *Vanellus malabaricus*

INTRODUCTION

Since last few years the rising antimicrobial resistant strains of bacteria along with increase in emerging and re-emerging pathogens of man as well as animals have gained attention of the researchers all over the world (Verma *et al*., 2007, 2012; Garmyn *et al*., 2011; Kumar *et al*., 2011, 2012). Among them, extended-spectrum beta-lactamases producing *E. coli*

(ESBL-*E. coli*) poses a major threat to man and animal, especially in the form of nosocomial infections (Guenther *et al*., 2011) and may cause bacteremia, wound infections, urinary tract infection, neonatal meningitis and gastrointestinal infections (Raina *et al*., 1999; Kumar *et al*., 2013). There are many reports that birds may act as reservoir of multi drug resistant (MDR) bacteria (Guenther *et al*., 2010) and play a crucial role in spread of antibiotic resistance (Garmyn *et al*., 2011). Mathur *et al*. (2002) reported 68% ESBL positivity rate in bacteria of enterobacteriacae family from India. Thus, global increasing trend in ESBL mediated resistance among bacteria particularly in *E. coli* has pulled the focus of researchers towards this public health issue. However, to the best of author's knowledge, there is no report for these in Yellow-wattled Lapwing (*Vanellus malabaricus*). In this context, the present study describes the possible cause of death in three chicks of yellow-wattled bird (*Vanellus malabaricus*); presence of ESBL producing *E. coli* in Yellow-wattled Lapwing and chances of dissemination of antibiotic resistant bacteria in natural environment.

MATERIALS AND METHODS

Three chicks were found on roadside to Veterinary University (DUVASU) campus, Mathura appeared to suffering with certain illness showing clinical signs such as disability to walk, difficult respiration and inability to eat and drink on the grounds near to premises, where these birds stay throughout year and probably breed also. These chicks died within few hours before any rehabilitation was started, except the attempts of feeding and providing drinking water. Then post-mortem examinations were conducted to know the possible cause of death.

Sample collection: Died chicks were cleaned with 70% alcohol and then opened aseptically to collect the tissues in sterile container in phosphate buffered saline (pH 7.4) and heart blood. Tissues were collected from heart, liver, lung and kidney. The collected tissues were immediately shifted to laboratory for further isolation of microbial agents.

Microbiological culture and identification: Microbiological isolation was attempted from tissues (lungs, heart, liver and kidney) and heart blood samples of dead chicks. Samples were inoculated onto nutrient agar, MacConkey Lactose agar (MLA), Sabouraud's Dextrose agar (SDA) and 5% chicken blood agar plates and incubated aerobically/microaerobically at 37°C for 24 h (Quinn *et al*., 2002). After 24 h incubation, the plates were observed for presence of bacterial and fungal colony. Lung tissues for mycoplasma isolation were homogenized in 1 mL PBS and then 100 μL of this solution was transferred into Pleuropneumonia like Organism broth (PPLO broth) enriched with horse serum and incubated at 37°C, 5% CO_2 for 48 h. (Rosengarten *et al*., 1994) afterwards. Samples from PBS (pH 7.4) were triturated, filter sterilized (0.22 micron syringe filter) and inoculated on Maiden Durby Bovine Kidney (MDBK) cell lines and incubated at 36°C, 5% CO_2 for 48h in CO_2 incubator for virus isolation.

Lung samples for the isolation of mycoplasma were cultured and observed according to the method described by Ter Laak *et al*. (1992). Small pieces from altered lungs were homogenized in PBS and 10% cell suspension was cultured. The inoculated tubes were incubated at 37°C and plated on days 3 and 7. The plates were incubated at 37°C in 5% CO_2 atmosphere and were examined under a stereomicroscope every 2 days. Blood samples collected aseptically were also inoculated in the similar pattern with the help of sterilized bacteriological loop.

Isolation and identification of *E. coli*: All the pink colonies suspected to be of *E. coli* were further re-streaked on Macconkey's Lactose Agar (MLA) and after incubation at 37°C for 24-48 h pink colonies from each MLA plate were picked up and streaked on Eosin Methylene Blue (EMB) agar for observing the metallic sheen. Bacterial isolates suggestive of *E. coli* on MLA and EMB were studied on the basis of their cultural, morphological and motility characteristics. Then biochemical studies of *E. coli* isolates were carried out as per the method of Cruickshank *et al.* (1975).

Assessment of pathogenicity: All the isolates were assessed for their pathogenicity by Congo red binding and production of hemolysins. For this the colonies confirmed biochemical, cultural and morphological basis were grown at 37°C for 24 h on Tryptic soy agar supplemented with 0.02% Congo red and 0.15% bile salt and 5% sheep blood agar, respectively. Positive colonies for Congo red binding appeared red in color whereas haemolytic isolates produced a clear zone of haemolysis in surrounding to colonies.

Antibiogram: *In vitro* antibiotic susceptibility testing of all the isolates of *E. coli* recovered during study was conducted against antibiotic discs Streptomycin (10 μg), Chloramphenicol (30 μg), Amikacin (30 μg), Ampicillin (10 μg), Cephalexin (30 μg), Tetracycline (30 μg), Gentamicin (10 μg), ciprofloxacin (5 μg) and Norfloxacin (10 μg) using disk diffusion method (Bauer *et al.*, 1966). The zone size around each antibiotic disc was elucidated as sensitive, intermediate or resistant according to NCCLS (2002).

ESBL determination by double disk method: The disk approximation method was used with the antimicrobial disks of cefpodoxime and clavulanic acid. A Mueller-Hinton agar plate was inoculated with a suspension made from an overnight blood agar culture of the isolates as recommended for a standard disk diffusion susceptibility test. Disks containing the standard cefpodoxime (10 μg) are placed 15 mm apart (edge to edge) and from an amoxicillin-clavulanic acid disk containing 10 μg of the latter compound as per the recommendation of (Coudron *et al.*, 1997) to have greater sensitivity. Following incubation for 16-20 h at 35°C, any enhancement of the zone of inhibition between a beta-lactam disk and that containing the beta-lactamase inhibitor is indicative of the presence of an ESBL was observed with all the precautions (Moland and Thompson, 1994).

RESULTS AND DISCUSSION

The Yellow-wattled Lapwing, Vanellus malabaricus, is a commonly observed bird in Indian subcontinent though some reports also indicates their rare appearances in India. These belong to the family Charadriidae and found in dry plains of sub continent even in the close vicinity of human populations. They are ground birds and their nest is a mere collection of tiny pebbles within which their well camouflaged eggs are laid. These lapwings breed in the dry season with peak breeding in March to May ahead of the monsoons (Jayakar and Spurway, 1965). The incubation period was 27-30 days. The chicks are nidifugous, leaving the nest shortly after hatching and following their parents to forage for food. These Yellow-wattled Lapwings are a common feature on the grounds near the university premises, here in Mathura, India.

In the present investigation, the absence of growth in SDA, PPLO broth and further in PPLO agar, ruled out the possibilities of fungal pathogens and mycoplasma infections, while the growth

on nutrient agar and MLA plates revealed bacterial growth. There were no changes in MDBK cell lines as well. The further confirmation on the basis of cultural, morphological and biochemical characters confirmed it as *E. coli*. On blood agar these isolate revealed a hazy wider zone of haemolysis and also produced red color colonies on tryptic soya agar enriched with Congo red dye. Antibiogram of these isolates revealed resistance against streptomycin, tetracycline, ciprofloxacin, norfloxacin, ampicillin and cephalexin. Intermediate sensitivity was observed with chloramphenicol whereas isolates were sensitive to amikacin and gentamicin. Moreover, double disk diffusion revealed the presence of ESBL producing character of these isolates.

Thus, ESBL producing *E. coli* might be the cause of death. The probable source of infection might be the feeding habit of bird like beetles, termites and other invertebrates picked from the ground. Moreover, the place where they have nested was also near to the place where the carcasses of dead animals were deposited at that time and from where the parents of these chicks might have received these pathogens which appeared to be the cause of their death.

Yellow-wattled Lapwings parents visit water and wet their breast feathers {"belly soaking"; they may stay for as much as 10 min to soak water (Maclean, 1974)} which is then be used to cool the eggs or chicks (Jayakar and Spurway, 1964), might be the source of infection due to presence of these pathogens in contaminated water. As *Escherichia coli* are an important pathogens group in community and hospital-acquired infections and it is unfortunate to see the increasing resistance against common among gram-negative bacteria and ultimately making empirical therapy decisions more difficult and confusing. Resistance patterns among gram-negative organisms not only include resistance to extended spectrum of cephalosporin and penicillin but also against the third generation of antibiotics (Motta *et al*., 2003). Murugan *et al*. (2011) have also reported difficulties in the treatment of food and water associated gastrointestinal diseases due to *E. coli* and this problem is compounded by the continued emergence of antibiotic resistance to a growing number of antibiotics i.e. carbenicillin, tetracycline, streptomycin (Walia *et al*., 2004), norfloxacin, amoxycillin, trimethoprim, nitrofurantoin (Goettsch *et al*., 2000), nalidixic acid, gentamicin, cefuroxime (Shehabi *et al*., 2004), ampicillin, ceftriaxone, ciprofloxacin, ceftazidime and cefotaxime (Patoli *et al*., 2010) etc. Increased resistance to antibiotics is a global problem and makes the treatment extremely difficult or virtually impossible in some instances (El-Astal, 2004). Similar to methicillin resistant staphylococcus extended spectrum beta Lactamases producing strains of Enterobacteriaceae have now a day among major emerging problems particularly in hospitalized as well as community based patients. Moreover, the infections due to ESBLs-producers mainly affecting a range of patients from uncomplicated urinary tract infections (UTI) to life threatening sepsis producing conditions (Bhattacharya, 2006). The status of ESBL producing *E. coli* is also alarming in India with the 68% ESBL positivity rate in Enterobacteriacae isolates (Mathur *et al*., 2002).

CONCLUSION

The level of increasing ESBL mediated resistance amongst *E. coli* isolates worldwide and particularly in developing countries is among major public health threats. Now the presence of these ESBL producing multi drug resistant α hemolytic *E. coli* in Yellow-wattled Lapwing has posed another challenge to public health.

ACKNOWLEDGMENTS

The authors are highly thankful to Manoj Kumar Gupta, Incharge, Department of Veterinary Microbiology and Immunology, Dean, College of Veterinary Sciences and Hon'ble Vice Chancellor,

Uttar Pradesh Pandit Deen Dayal Upadhaya Pashu Chikitsa Vigyan Vishwavidhyalaya Evam GoAnusandhan Sansthan, Mathura, India for providing all the necessary facilities and supports to conduct this study.

REFERENCES

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bhattacharya, S., 2006. ESBL-from petri dish to the patient. Indian J. Med. Microbiol., 24: 20-24.

Coudron, P.E., S.E. Moland and C.C. Sanders, 1997. Occurrence and detection of extended-spectrum β-lactamases in members of the family Enterobacteriaceae at a veterans medical center: Seek and you may find. J. Clin. Microbiol., 35: 2593-2597.

Cruickshank, R., J.P. Duguid, B.P. Marmion and R.H.A. Swain, 1975. Medical Microbiology. 12th Edn., Churchill, Livingstone, Edinburgh, UK pp: 356.

El-Astal, Z., 2004. Bacterial pathogens and their antimicrobial susceptibility in Gaza strip, Palestine. Pak. J. Med. Sci., 20: 365-370.

Garmyn, A., F. Haesebrouck, T. Hellebuyck, A. Smet, F. Pasmans, P. Butaye and A. Martel, 2011. Presence of extended-spectrum β-lactamase-producing *Escherichia coli* in wild geese. J. Antimicrob. Chemother., 66: 1643-1644.

Goettsch, W., W. Van Pelt, N. Nagelkerke, M.G.R. Hendrix and A.G.M. Buiting *et al*., 2000. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. J. Antimicrob. Chemother., 46: 223-228.

Guenther, S., C. Ewers and L.H. Wieler, 2011. Extended-spectrum β-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front. Microbiol., Vol. 2. 10.3389/fmicb.2011.00246

Guenther, S., M. Grobbel, A. Lubke-Becker, A. Goedecke, N.D. Friedrich, L.H. Wieler and C. Ewers, 2010. Antimicrobial resistance profiles of *Escherichia coli* from common European wild bird species. Vet. Microbiol., 144: 219-225.

Jayakar, S.D. and H Spurway, 1964. Lapwings wetting their breasts. Newslett. Birdwatchers, 4: 5-5.

Jayakar, S.D. and H. Spurway, 1965. The yellow-wattled lapwing *Vanellus malabaricus* (Boddaert), a tropical dry-season nester. II. Additional data on breeding biology. J. Bombay Nat. Hist. Soc., 62: 1-14.

Kumar, A., A.K. Verma and A. Rahal, 2011. *Mycoplasma bovis*, a multi disease producing pathogen: An overview. Asian J. Anim. Vet. Adv., 6: 537-546.

Kumar, A., A.K. Verma, S. Malik, M.K. Gupta, A. Sharma and A. Rahal, 2013. Occurrence of extended spectrum β-lactamases producing alpha hemolytic *Escherichia coli* in Neonatal diarrhea. Pak. J. Biol. Sci., (In Press)

Kumar, R., A.K. Verma, A. Kumar, M. Srivastava and H.P. Lal, 2012. Prevalence and antibiogram of campylobacter infections in dogs of Mathura, India. Asian J. Anim. Vet. Adv., 7: 434-440.

Maclean, G.L., 1974. Belly-soaking in the Charadriiformes. J. Bombay Nat. Hist. Soc., 72: 74-82.

Mathur, P., A. Kapil, B. Das and B. Dhawan, 2002. Prevalence of extended spectrum β-lactamase producing gram negative bacteria in a tertiary care hospital. Indian J. Med. Res., 115: 153-157.

Moland, E.S. and K.S. Thompson, 1994. Extended-spectrum β-lactamases of Enterobacteriaceae. J. Antimicro. Chemother., 33: 666-668.

Motta, R.N., M.M. Oliveira, P.S. Magallaes, A.M. Dias, L.P. Aragao, A.C. Forti and C.B.M. Carvallo, 2003. Plasmid mediated extended spectrum β-lactamase producing strains of Enterobacteriaceae isolated from Diabetes foot infection. Braz. J. Infect. Dis., 2: 129-134.

Murugan, S., Jayanthi, P.U. Devi and P.N. John, 2011. Prevalence and Antimicrobial susceptibility pattern of extended spectrum β-lactamases producing *Escherichia coli* in Diabetic Foot Infection. Res. J. Microbiol., 6: 609-617.

NCCLS, 2002. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard. 2nd Edn., National Committee for Clinical Laboratory Standards, Wayne, PA., USA., ISBN: 1-56238-461-9.

Patoli, A.A., B.B. Patoli and V. Mehraj, 2010. High prevalence of multi-drug resistant *Escherichia coli* in drinking water samples from Hyderabad. Gomal J. Med. Sci., 8: 23-26.

Quinn, P.J., B.K. Markey, M.E. Carter, W.J.C. Donnelly and F.C. Leonard, 2002. Veterinary Microbiology and Bacterial Disease. Blackwell Science, London, UK., Pages: 648.

Raina, P.S., F.L. Pollari, G.F. Teare, M.J. Goss, D.A. Barry and J.B. Wilson, 1999. The relationship between *E. coli* indicator bacteria in well-water and gastrointestinal illnesses in rural families. Can. J. Public Health, 90: 172-175.

Rosengarten, R., A. Behrens, A. Stetefeld, M. Heller and M. Ahrens *et al*., 1994. Antigen heterogeneity among isolates of *Mycoplasma bovis* is generated by high-frequency variation of diverse membrane surface proteins. Infect. Immun., 62: 5066-5074.

Shehabi, A.A., A.M. Mahafzah and K.Z. Al-Khalili, 2004. Antimicrobial resistance and plasmid profiles of urinary *Escherichia coli* isolates from Jordanian patients. Eastern Mediterranean Health J., 10: 322-328.

Ter Laak, E.A., G.H. Wentink and G.M. Zimmer, 1992. Increased prevalence of *Mycoplasma bovis* in the Netherlands. Vet. Q., 14: 100-104.

Verma, A.K., A. Kumar, K. Dhama, R. Deb, A. Rahal, Mhima and S. Chakraborty, 2012. Leptospirosis-Persistence of a dilemma: An overview with particular emphasis on trends and recent advances in vaccines and vaccination strategies. Pak. J. Biol. Sci., 15: 954-963.

Verma, A.K., D.K. Sinha and B.R. Singh, 2007. Salmonella in apparently healthy dogs. J. Vet. Public Health, 5: 37-39.

Walia, S.K., A. Kaiser, M. Parkash and G.R. Chaudhry, 2004. Self-transmissible antibiotic resistance to ampicillin, streptomycin and tetracyclin found in *Escherichia coli* isolates from contaminated drinking water. J. Environ. Sci. Health A, 39: 651-662.