Explor. Anim. Med. Res.,
Vol.3, Issue - 2, 2013, p. 165-168

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

CHARACTERIZATION AND ANTIBIOGRAM OF ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM POULTRY

M. Sarkar, J.P. Roy and K. Batabyal*

ABSTRACT: One hundred sixty two samples from different poultry farms of West Bengal, India were screened for the presence of pathogenic *Escherichia coli* and 109 (67.3%) were found positive. Out of forty six faecal samples from ailing birds suffering from acute colibacillosis, thirty one *i.e.* 67.2% were positive whereas postmortem sample of intestines (62) and liver tissues (54) revealed approx 72.6% and 61.1% positivity for *E. coli*. Biochemical characteristic of the isolates were positive to indole, MR, nitrate and non-reactive to VP, citrate & urease test. In serotyping of the *E. coli* isolates mostly revealed O2, O8, O9, O19, O37, O47, O55, O69, O86, O101, O103, O109, O133, O151 and O173. The serotypes *viz.* O2, O8, O9, O55, O101 and O133 showed acute pathogenicity in swiss mice followed by O19, O37, O47, O69, O86, O103, O109, O151 and O173 as moderately pathogenic serotypes. Among the antimicrobial drugs tested, the sensitive drugs were cefixime (93.6%), enrofloxacin (91.8%), nitrofurantoin (88.1%) and azithromycin (85.3%). The resistant drugs were tetracycline (100%), nalidixic acid (97.2%), metronidazole (92.6%), penicillin G (88.9%), gatifloxacin (77.9%) and bacitracin (76.2%) .

Key words: *Escherichia coli*, Poultry, Characterization, Pathogenicity, Antibiogram

INTRODUCTION

The organism *Escherichia coli* (*E.coli)* are present in nature and normal inhabitants of intestinal tracts of animals and man. In poultry, *E.coli* causes colibacillosis, colisepticaemia, coligranuloma, pericarditis, omphalitis, air saculitis etc (Orden *et al.*1999). Severe outbreaks of this infection in poultry cause high morbidity and mortality resulting farmer's economic loss (Zhang *et al*. 1996). Factors responsible for the spread of this infection are contaminated food, sewage and water. The epidemiological studies and complete identification on the basis of different O antigens of *E. coli i.e* serological typing was carried out in the institute of NSEC, CRI, Kasauli, H.P. The present investigation was aimed to identification, characterization and serological typing of *E. coli* associated with various pathological conditions in poultry along with study of pathogenicity in mice followed by antibiogram with various antibiotics which have been widely used for treatment and control of *E. coli* infections.

MATERIALS AND METHODS

In this study, 162 samples were collected from

Department of Veterinary Microbiology, F/VAS,West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata – 700 037, West Bengal, India.
***** *Corresponding Author.*

for isolation and identification of pathogenic *E. coli* from different Poultry farms of West Bengal, India. The samples include faecal samples (46) from birds suffering from acute colibacillosis and post-mortem samples *viz.* intestines (62) and liver (54) from dead birds with the lesions of colibacillosis, pericarditis, air saculitis, omphalitis etc.

The faecal samples were mixed & enriched directly in separate tubes with 10ml sterile EC broth and incubated at 37°C for 18-20 hours. The visceral organs were enriched by mixing 5ml of tissue suspension with 10ml of sterile EC broth and incubated. All samples after enrichment were streaked on MacConkey's agar plates and incubated for overnight at 37°C. Tentative pink colonies on MacConkey's agar were re-cultured on Eosin Methylene Blue (EMB) agar for purification.

Dark chocolate colour colonies with typical 'metallic sheen' were considered for morphological and biochemical characterization with different tests, *viz.* IMVIC reactions, TSI agar test, H_2S production test, nitrate reduction test & urease test as per Buxton and Fraser (1977). Tentatively positive *E. coli* strains were serotyped serologically at NSEC, CRI, Kasauli after primarily confirmation.

The pathogenicity of the selected isolates was tested in swiss albino mice as per Gupta and Singh (1969). Five ml young broth culture of each *E. coli* isolate was inoculated via I/P route @ 0.2ml into a batch of 6 mice (6-8 weeks old) and observed for any abnormality/ disease symptom or death. Control group mice were injected with sterile NSS. Re-isolation of *E. coli* from the infected viscera of dead mice was tried for confirmation.

Antibacterial sensitivity test of selective serotypes against 10 different antibacterials, namely bacitracin, nalidixic acid, tetracycline, nitrofurantoin, cefixime, gatifloxacin, azithromycin, enrofloxacin, penicillin G and metronidazole were performed by disc diffusion technique as per Bauer *et al.* (1966). After overnight incubation at 37°C, the zones of inhibition were measured and interpreted as per the given zone diameter in the interpretative chart.

RESULTS AND DISCUSSION

One hundred and nine (67.3%) positive *E. coli* isolates were obtained from the test samples (162) collected from ailing and dead poultry birds in this study. The intestinal samples yielded the highest positivity of *E. coli i.e* 45 (72.6%) followed by faecal samples (31 *i.e* 67.2%) and liver samples (33 *i.e* 61.1%). The earlier reports by previous workers like Sarkar and Soman (1992), Chakraborty and Nag (1998) etc. were in accordance with this report of positivity from poultry. The highest positivity of *E. coli* in intestines and in faeces revealed the gut acting habit of *E. coli* which was also supported by Sharma *et al.* (1987) and Chakraborty and Nag (1998).

All typical isolates with pink colonies on MacConkey's agar showed dark chocolate colonies with characteristic 'metallic sheen' on EMB agar. These isolates were actively motile and showed typical reactions during biochemical characterization with positive results on indole test, MR test and nitrate reduction test and were negative to VP test, citrate utilization test, urease test & H_2S production as per Buxton and Fraser (1977). They produced both yellowish acid butt and acid slant on TSI agar. These reports are in accordance with that of earlier workers like Chakraborty and Nag (1998), Sharma *et al.* (1995) and Rajeswari *et al.* (1992).

Serological typing at NSEC, CRI, Kasauli of these isolates revealed serotypes like O2, O8

Table 1: Details of results of antimicrobial sensitivity test

Sl. No.	Name of antimicrobial agents`	Strength (mcg)	Total isolates tested	Resistant		Intermediate Sensitive		sensitive	
				No.	%	No.	%	No	.%
1	Bacitracin (B)	10	109	83	76.2	24	22.0	2	1.8
2	Nalidixic acid (NA)	30		106	97.2	3	2.8	-	-
3	Tetracycline (TE)	30		109	100	-		-	-
4	Nitrofurantoin (NIT)	100		-	-	13	11.9	96	88.1
5	Cefixime (CFM)	05		-	-	7	6.4	102	93.6
6	Penicillin G (PG)	10		97	88.9	12	11.1	-	-
7	Gatifloxacin (GAT)	05		85	77.9	20	18.4	4	3.7
8	Azithromycin (AZM)	15		4	3.7	12	11.0	93	85.3
9	Enrofloxacin (EX)	05		2	1.8	7	6.4	100	91.8
10	Metronidazole (MT)	04		101	92.6	8	7.4	-	-

(9 isolates each), O9, O55, O69 (8 isolates each), O19, O101, O109, O151(7 isolates each), O33, O41 (6 isolates each), O47, O86, O133 (4 isolates each), O107, O173 (3 isolates each), O18 & O103 (1 isolate each). Five isolates were rough and two were untypable in this study. Previous workers like Mehrotra *et al.* (1984), Sharma *et al.* (1995) and Mohanty *et al.* (1979) were also reported the presence of these serotypes from poultry samples in their study.

The serotypes *viz.* O2, O8, O9, O55, O101 and O133 which caused 100% mortality in mice within 24hrs during pathogenicity test were considered highly pathogenic, but the serotypes *i.e.* O18, O33, O47, O86, O103, O109 and O151 were revealed to be moderately pathogenic with 75% death of mice within 48hrs of inoculation. The serotypes *i.e.* O19, O69, O107 and O173 were mild pathogenic to mice. Congestion and discolouration of viscera were noticed as the gross pathological changes on postmortem and *E. coli* from the viscera of the dead mice were re-isolated. These reports were in agreement with earlier reports by Sharma *et al.* (1995), Mishra (1991) and Mukherjee *et al.* (1997).

In antimicrobial sensitivity test of all isolates, it was noticed that cefixime (93.6%), enrofloxacin (91.8%) nitrofurantoin (88.1%) & azithromycin (85.3%) were highly sensitive against these pathogens which were also supported by Zhang *et al.* (1996). Resistant drugs like tetracycline (100%), nalidixic acid (97.2%), metronidazole (92.6%), penicillin G (88.9%), gatifloxacin (77.9%) and bacitracin (76.2%), [Table 1] of these isolates were in accordance with earlier reports by Osmani *et al.* (1992), Mishra (1991) and Sharma *et al.* (1995).

CONCLUSION

Sixty seven percent samples collected from ailing and dead poultry birds were found positive for presence of *E. coli*. Intestinal samples were the highest in positivity followed by faecal and liver samples. Positive isolates showed typical

results in morphological and biochemical characterization. The detected serotypes were O2, O8, O9, O19, O47, O55, O69, O101, O107, O109, O133, O155 and O173. The serotypes like O2, O8, O9, O55, O101 and O133 were highly pathogenic to swiss mice. Antimicrobial drugs *i.e.* cefixime, enrofloxacin, nitrofurantoin and azithromycin were sensitive but tetracycline, nalidixic acid, gatifloxacin and bacitracin were resistant against these isolates.

ACKNOWLEDGEMENT

The authors were very much grateful to the Vice-Chancellor, The Director of Research, The Dean F/VAS, West Bengal University of Animal and Fishery Sciences, Kolkata for providing facilities for this research work.

RFERENCES

Bauer AW, Kirby MM, Sherris JC and Truck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American J. Clin. Pathol.* 45: 493 - 496.

Buxton A and Fraser G. (1977). *Animal Microbiology.* Vol. 1. Blackwell Scientific Publications. Edinburg. p. 157 - 158.

Chakraborty D and Nag NC. (1998). Some observations on the colicinogenicity and enteropathogenicity of *Escherichia coli* associated with gastroenteritis in calves. *Indian J. Anim. Hlth.* 37(1): 17 - 20.

Gupta RN and Singh CM. (1969). Studies on *Escherichia coli* from cases of colisepticaemia of poultry in India. *Indian J. Anim. Sci.* 39: 231 - 241.

Mehrotra P, Khotia R and Mehrotra P. (1984). Occurrence of serotypes and antibiotic resistance in *Escherichia coli* isolates from different species. *Indian J. Anim. Sci.* 54: 396-399.

Mishra KC.(1991). *Escherichia coli* strains from different sources in Assam. *Indian J. Anim. Hlth.* 30: 23 - 28.

Mohanty P, Kulshrestha S and Sharma T. (1979). Studies on *E. coli* serogroups from enteritis cases of poultry. *Indian J. Poult. Sci*. 74(2): 78 - 85.

Mukherjee BN, Mandal D and Mishra SK. (1997). Pathogenicity of *Escherichia coli* isolated from chicks in laboratory animals. *Indian J. Anim. Hlth.* 36: 151 - 156.

Orden JA, Ruiz JA, Cid D, Garcia S and Fuente R de la.(1999). Prevalence and charac-teristics of necrotoxigenic *Escherichia coli* strains isolated from diarrhoeic calves. *Vet. Microbiol.* 66(4): 265 - 273.

Osmani AS, Sinha RP and Soman JP.(1992). Studies on bacteria isolated from enteric goats. *Indian J. Vet. Med.* 12(1): 31 - 32.

Rajeswari K, Reddy R, Sharma BD and Dhanalakshmi BR.(1992). Normal aerobic bacterial flora of respiratory tract, intestinal tract, oviducts of Japanese quails. *Indian Vet. J.* 69(10): 880 - 883.

Sarkar SK and Soman JP.(1992). Identification and pathogenicity of bacterial agents isolated from piglet enteritis. *Indian J. Anim. Sci.* 62(50): 391 - 397.

Sharma DK, Sambyal DS and Sharma S.(1987). *Escherichia coli* serotypes in domestic fowls of Punjab. *British Vet. J.* 143(3): 273 - 277.

Sharma DK, Singh N and Joshi DV.(1995). *Escherichia coli* in milk, meat and meat products: isolation, characterization, antibiogram and zoonotic significance. *J. Food Sci. Technol*. 32(50): 409 - 412.

Zhang S, Zhao Y, Zhao Z and Kang Z.(1996). Drug sensitivity test on pathogenic *Escherichia coli* from chickens in Guang, Zhong, Shaonxi. *Chinese J. Vet. Sci. Tech.* 26(1): 29 - 30.

***Cite the article as:** Sarkar M , Roy JP , Batabyal K.(2013). Characterization and antibiogram of enteropathogenic *Escherichia coli* isolated from poultry. *Explor. Anim. Med .Res.* 3(2): 165-168.