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Research Article

IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* GALLINARUM ISOLATED FROM FOWL TYPHOID OUTBREAK IN BACKYARD *VANARAJA* FOWL

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ABSTACT: From a disease outbreak among *Vanaraja* fowl, an indigenous Indian Breed reared by backyard system in Jhargram, West Bengal, *Salmonella* Gallinarum was isolated and characterised. The outbreak occurred among 6-8 day old chicks. A total of 150 birds died in a span of 5 days. *Salmonella* Gallinarum were identified and confirmed by standard bacteriological methods and presence of invasion (*inv*A) gene was detected by PCR. The isolates were susceptible to 15 common antimicrobials *in vitro*. Although chemotherapy may be effective, outbreaks of fowl typhoid in backyard poultry warranted precise control policy.

Key words: Backyard poultry, Fowl typhoid, *Salmonella* Gallinarum, West Bengal.

INTRODUCTION

Backyard poultry contributes a major share to the egg production of West Bengal and supports livelihood to low income group people. Due to lack of proper immunisation, backyard poultry production is under serious threat from various diseases. Fowl typhoid (FT) caused by *Salmonella* Gallinarum, is an acute septicaemic disease of poultry. The disease is generally associated with high mortality in chicks, and birds recovered from clinical infection become chronic carriers of the disease. The disease also causes heavy economic loss through death and decreased egg production in commercial poultry. From infected layer birds, the pathogen is transmitted vertically via eggs or laterally by direct contact or mechanical transfer by people, equipments, feed or water (Quinn *et al*., 2011). Despite eradication in some parts of the world through improved surveillance and culling, outbreak reports are still common, both in commercial and backyard poultry in developing countries (Barrow and Freitas Neto, 2011).

Salmonella Gallinarum is the predominant

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Table 1: Biochemical reactions of *Salmonella* sp. isolates from backyard poultry.

Character/test	Reactions
Growth on MacConkey's agar	Pale yellow colonies
Growth on BGA	Pink colonies
Indole	-ve
Methyl Red	+ve
Vogues-Prausker	-ve
Oxidase	-ve
Catalase	+ve
Citrate utilisation	+ve
Nitrate reduction	+ve
O/F test	fermentative
Urease	-ve
TSI slant	Alkaline slant, acid butt
Fermentation of sugars	
Maltose	+ve
Dulcitol	+ve
Glucose	+ve
Xylose	+ve
Rhamnose	+ve
Salicin	-ve
lactose	-ve
Motility	-ve

serovar causing high mortality in most of the poultry rearing states like Haryana (Aorora *et al.*, 2013; Kumar *et al*., 2012), Kerala (Ambily and Mini, 2014), Karnataka, Maharastra and Taminadu (Prakash *et al.,* 2005) and West Bengal (Chakraborty *et al*., 1999). However, unlike in organised intensive poultry farms, FT have been meagrely studied in backyard poultry. The objectives of the present work were characterisation and antimicrobial susceptibility study of *Salmonella* Gallinarum isolated in a FT outbreak of backyard poultry.

MATERIALS AND METHODS

The study was conducted after a reported disease outbreak in Vanaraja fowl (n=1200) of 6-8 day old in Jhargram, West Bengal, India. As per the report, 150 chicks died in a span of 5 days. The affected animals showed anorexia, depression, diarrhoea and death. Post mortem examination was performed and organ samples like spleen, liver with gall bladder and heart blood were collected aseptically. Isolation of probable causative agents was done using standard bacteriological methods (OIE, 2012). The organ samples were streaked on MacConkey lactose agar for primary isolation of *Salmonella* Gallinarum and then on Brilliant green agar (BGA, HiMedia, India). Identification of the isolates was performed by employing biochemical tests *viz*. catalase, oxidase, O/F test, motility test using motility medium, triple sugar iron agar (TSI), urease, nitrate reduction, indole, methyl red, Voges Proskauer, citrate (IMViC) and sugar fermentation tests. The isolates were also examined for presence of invasion (*inv*A) gene of *Salmonella* sp. by PCR using forward primer 5'- GTGAAATTATCGCCACGTT CGGG CAA-3'and reverse primer 5'-TCAT CGCACCGTCAAAGGAACC-3' (Oliveira *et al*., 2002).

In vitro susceptibility of the isolates to 15 antimicrobial agents was determined by the disc diffusion technique (Bauer *et al.*, 1966). Fifteen antimicrobial discs tested were amikacin (30 µg), amoxicillin- clavulanic acid (20/10 µg), ampicillin–salbactum (10/10 µg), azithromycin

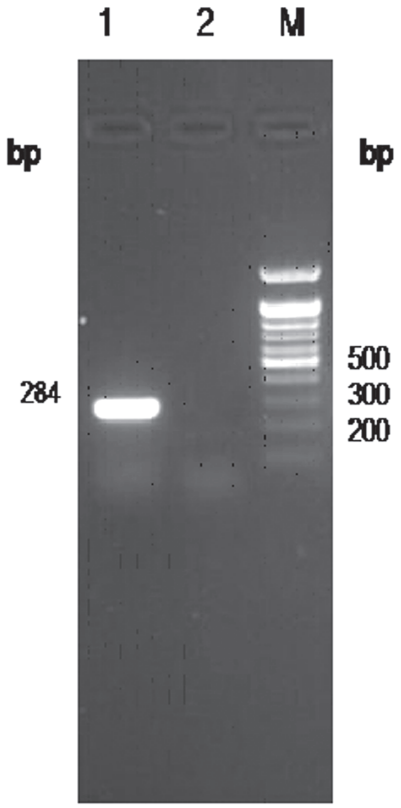

Fig. 1. Detection of Salmonella sp. by *inv*A gene PCR
[Lane 1: Representative sample of Salmonella sp. isolates; Lane 2: Negative control; Lane M: 100 bp DNA ladder].

(15µg), ceftriaxone (30µg), co-trimoxazole (25µg), chloramphenicol (30µg), ciprofloxacin (5µg), colistin (10 µg), doxycycline hydrochloride (30 µg), enrofloxacin (10 µg), erythromycin (15µg), streptomycin (300µg), tetracycline (30 µg) and gentamicin (10 µg). The discs were procured from HiMedia Laboratories, Mumbai. The diameter of zone of inhibition was measured and the sensitivity pattern of the test organism was determined according to Clinical and Laboratory Standard Institute (CLSI, 2013).

RESULTS AND DISCUSSION

Fowl typhoid outbreaks are the commonest form of salmonellosis in poultry in India (Kumari *et al*., 2013). Chakraborty *et al*. (1999) also reported high prevalence (60%) of *Salmonella* Gallinarum in chicks as early as 2 weeks of age. In present study, enlarged bronze- coloured liver with several discrete necrotic foci and enteritis were prominent gross lesions in affected chicks. The organism produced lactose non-fermenting pale colonies on MacConkey's agar and pale red colonies on BGA medium. Gram negative small bacilli were found by Gram staining. Biochemical characters of the isolates were similar to that of typical *Salmonella* sp., and were negative to indole, Voges Proskauer, oxidase, urease tests and showed positive reaction to the tests of methyl red, catalase, citrate utilisation and nitrate reduction. In TSI agar, the organism produced H_2S with alkaline slant and acid butt (Table 1). Four isolates were identified as *Salmonella enterica* biovar Gallinarum based on characteristic sugar fermentation reactions *viz*. fermentation of glucose with no gas, maltose, dulcitol, rhamnose, xylose and negative to salicin. Furthermore, the isolates were also confirmed by presence of invasion (*inv*A) gene in PCR (Fig. 1), which is present in all *Salmonella* organisms.

In the *in vitro* sensitivity tests, all the isolates from backyard poultry were found 100% sensitive to 15 antimicrobials. However, antimicrobial resistance phenomenon has been recorded in isolates of commercial broiler and layer fowls in previous studies. *Salmonella* spp. were found to be highly sensitive to amoxicillin, amoxicillin-sulbactum, enrofloxacin and doxycycline (91.66%) in Haryana (Kumari *et al*., 2013). Taddle *et al*.(2012) observed

variability in antimicrobial sensitivity among *Salmonella* spp. and sensitivity of *Salmonella* Gallinarum to amoxicillin / clavulanic acid and gentamicin were 93.3%, followed by ciprofloxacin, ofloxacin, colistin and cotrimoxazole (88.8%). Arora *et al.*(2013) in their study of 126 *Salmonella* Gallinarum, 15 *Salmonella* Enteritidis and 9 *Salmonella* Typhimurium observed that *Salmonella* spp. were most sensitive to gentamicin (76%) followed by amikacin (72%) and kanamycin (71%). Rai *et al.*(2004) noticed that susceptibility of *Salmonella* Gallinarum isolates were highest to chloramphenicol followed by ampicillin, streptomycin, tetracycline, nitrofurantoin, cefalexin, neomycin and enrofloxacin. High antimicrobial resistance of *Salmonella* Gallinarum to amoxiciilin and furazolidone (Chakraborty *et al.,* 1999) and 84% to nalidixic acid (Kumari *et al*., 2013) have been recorded earlier. Lee *et al.* (2003) observed reduced susceptibility of 46 *Salmonella* Gallinarum isolates to ampicillin (13.0%), gentamicin (43.4%), kanamycin (69.6%), enrofloxacin (6.5%), ciprofloxacin (10.9%), norfloxacin (52.5%) and ofloxacin (82. 6%) in Korea. *Salmonella* spp. were found resistant to norfloxacin, amoxicillin, ampicillin, nitrofurantoin and chloramphenicol in Nigeria (Musa *et al*., 2014). None of the strains we isolated were fully resistant to erythromycin and streptomycin, distinguishing them from the vaccine strain, SG 9R (Lee *et al*., 2013). In the current study, we found limited drug resistance in *Salmonella* Gallinarum isolates which may be attributable to the minimum exposure to antimicrobials in these backyard fowls reared in rural area. However, antimicrobial agents are reported to be effective in reducing mortality but do not completely eliminate *Salmonella* from a flock and recovered birds often becomes resistant carriers (Prakash *et al.*, 2005).

Free range system of management in backyard poultry rarely have full proof adaptation of hygienic and bio-security measures. Free-range rearing of both layer and broiler birds, now adapted following animal welfare issues in some countries, also increases risk of contamination. Although susceptibility to FT varies in birds of all ages depending on the bacterial virulence and genetic background of host (Barrow and Freitas Neto, 2011), backyard Vanaraja fowls seem to be vulnerable to FT infection. Zero tolerance in sero-monitoring of the parent stock and integrated hatchery management practices may be helpful in controlling vertical transmission of the disease.

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