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A biological tool to combat against multidrug-resistant *Salmonella* isolated from poultry of Chittagong City, Bangladesh

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ABSTRACT

Thirty two strains of *Salmonella* were isolated from the samples collected from different poultry farm of Chittagong City. Isolated organisms were then subjected to antibiotic sensitivity test against seven different standard antibiotics. Most of the strains tested were resistant to four antibiotics; 93.75% were resistant to co-trimaxazole, while 90.62% were resistant to cephotaxime, nalidixic acid and Tetracycline each. In this study, ciprofloxacin and gentamicin were found to be the most potent drugs, (78.125%) were sensitive to Ciprofloxacin & (78.125%) were sensitive to gentamicin. Antimicrobial activity of bark extract of *T. arjuna* against 13 selected isolates of *Salmonella* were then determined. During the course of the anti-microbial screening it was found that among the 13 selected isolates, *Salmonella* (L3-B1), *Salmonella* (L5-X4), *Salmonella* (F-X2) and *Salmonella* (S2-B1) showed good sensitivity to crude extract of *T. arjuna.*

Key words: *Salmonella*, Chittagong City, Poultry, Antibiotic Resistant, *T. arjuna.*

INTRODUCTION

Salmonella are known to cause diseases in humans, animals, and birds (especially poultry) worldwide. The principal habitat of the salmonellae is the intestinal tract of humans and animals. One of the constraints in the poultry farms in Bangladesh is the outbreak of infectious diseases. *Salmonella* infection is one of the most important bacterial diseases in poultry causing heavy economic loss through mortality and reduced production (Haider *et al*., 2004). Avian salmonella infection may occur in poultry either in acute or chronic form by one or more member of genus *Salmonella*, under the family Enterobacteriaceae (Hofstad *et. al*., 1992).

Evolution of antibiotic resistant bacteria is of serious concern now-a-days because of imbalanced uses of antimicrobial agents. Resistant organisms are capable to adjust in some way that reduces or eliminates the efficiency of drugs designed to prevent infections. If a bacterium carries several resistant genes, it is called multiresistant or, informally, a superbug. The extensive use of antimicrobials in human and animals has led to an increase in bacterial multidrug resistance among several bacterial strains. This phenomenon of multiple resistance represents a worldwide problem both for veterinary and public health sectors (White *et al.*, 2001). The routine practice of giving anti-microbial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth is an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to human through the food chain (Salehi *et al.*, 2005). Most of the infections with antibiotic resistant *Salmonella* are acquired by eating contaminated foods of animal origin (Fey *et al*., 2000).

The main objective of this research was to determine the multidrug-resistant status of *Salmonella* isolated from poultry of Chittagong City, Bangladesh and to find out a biological tool to combat against multidrug-resistant *Salmonella.*

MATERIALS AND METHODS

Collection of poultry samples:

Samples were collected from different poultry farms of Chittagong City. Liver, kidney, flesh, heart, albumin, yolk, spleen and lung samples of the chickens died from salmonella infection were subjected to investigation.

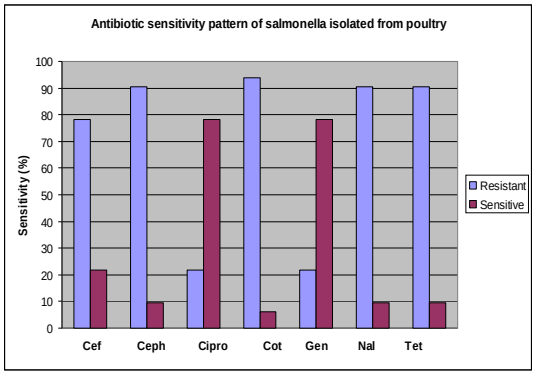

Fig-1: Graphical representation of antibiotic pattern of Salmonella isolated from poultry.

After collection, samples were placed in a sterile ice-bag, transported to the laboratory of BCSIR, Chittagong and maintained a temperature ranging

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from 4°C to 6°C. Microbiological examination was done promptly to avoid undesirable changes.

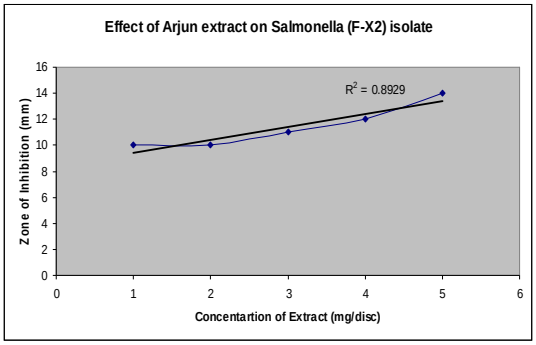

Fig-2: Effect of *T. arjuna* extract on F-X2 strain of resistant salmonella

Isolation of *Salmonella*:

Samples were aseptically cultured into Selenite Cystein Broth (Hi media, India) and incubated at 37ºC for 18-24 hours. After incubation, a loopful of each broth was streaked onto Salmonella-Shigella agar (SS agar). Xylose Lysine Deoxycholate agar (XLD agar) and Brilliant Green agar were also used as selective media for primary isolation of Salmonella. (Salehi *et al.*, 2005). Biochemical tests were performed for identification of the organism including: Triple sugars iron test, Urease test, Motality Indole urea test, Citrate utilization test Lactose fermentation test, Methyl Red test and Voges Proskauer test (VP)**.** The organisms were then subjected to gram staining and observed under microscope.

Collection and extraction of plant material:

The bark of *T. arjuna* was collected in fresh condition from Chittagong Hill tracts. The cleaned samples were cut into small pieces (1-2 cm) and dried in air. The samples were ground to fine powder mechanically and the dried powder was kept steeped for 72 hours in ethanol.

The extract thus obtained was filtered, subjected to rotary vacuum evaporator at 50ºC and concentrated to gummy material under reduced pressure. The gummy material was then collected in a small vial and then dried. Thus crude extracts were obtained**.**

Table-1: Percentage of Salmonella obtained from different organs of poultry

Name of Organ	Number of sample	Total isolates	Total Salmonella	Percent
1. Liver	5	45	17	37.77
2. kidney	1	1	1	100
3. Flesh	1	5	3	60
4. Heart	1	4	3	75
5. Yolk	1	5	0	0
6. Albumin	1	4	0	0
7. Lung	2	13	4	30.76
8. Spleen	2	8	4	50

Antibiotic sensitivity test against standard antibiotics:

For *in vitro* susceptibility test, the isolated organisms were subjected to seven standard antibiotics. The disc diffusion method was followed to determine susceptibility of the *Salmonella* isolates (Coyle, 2005)**.** The bacterial suspension turbidity was adjusted to McFarland standard number 0.5, in Mueller Hinton Broth (Hi-media, India). With a sterile cotton swab bacterial culture was streaked on Mueller Hinton Agar plate (Hi-media, India). Commercial antibiotic discs containing single concentration of each antibiotic were then placed on the inoculated plate surface. The zone of inhibition of growth around each disc after incubation at 37ºC was measured in millimeters.

Seven standered antibiotic discs (Oxoid, England) were used. Names of the antibiotic discs and their concentrations were as below: Ceftriaxone (30 µg), Cephotaxime (30 µg), Ciprofloxacin (5 µg), Co-trimaxazole (25 µg), Gentamicin(10 µg), Nalidixic Acid (30 µg) and Tetracycline (30 µg).

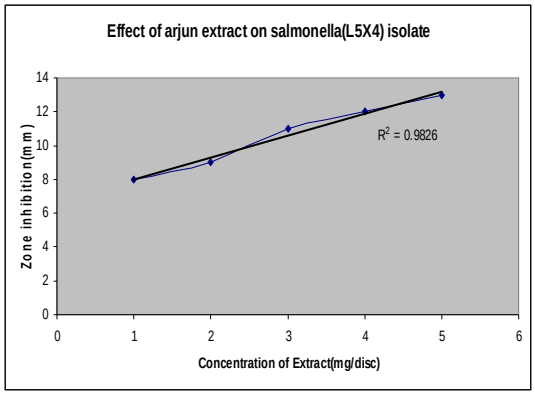

Fig-3: Effect of *T. arjuna* extract on L5X4 strain of resistant Salmonella

Determination of antibacterial activity of *Terminalia arjuna* crude extracts:

In vitro sensitivity of the 13 selected *Salmonella* isolates to the crude extract of *T arjuna* was determined by disc diffusion method (Bauer *et al.*, 1966). Dried and sterilized paper discs were treated separately with desired concentration of previously prepared ethanolic solution of the crude extract using a micropipette, dried in air under aseptic conditions and placed at equidistance in a circle on the seeded plate. The concentrations of crude extract used per disc were 1, 2, and 3 mg. These plates were kept for

4-6 hours at low temperature, so that the test material was diffused from disc to the surrounding medium by this time. The plates were then incubated at 37ºC for 24 hours.

RESULTS

Eighty five (85) organisms were isolated from different poultry samples viz. liver, heart, spleen, kidney, lung, albumin, yolk and flesh. Maximum *salmonella* were obtained from liver. There were no *Salmonella* found in eggs (Table-1).

Antibiotic sensitivity pattern:
Thirty two strains of S*almonella* were subjected to antibiotic sensitivity test against seven commonly used antibiotics belonging to different groups.

Table-2: Antimicrobial activity of *T. arjuna* against antibiotic resistant *Salmonella.*

Name of the organism	Zone of inhibition (mm)				
	1 mg/disc	2 mg/disc	3 mg/disc	4 mg/disc	5 mg/disc
Salmonella (L1B2)	8	10	10	11	12
Salmonella (L3B1)	9	11	11.5	12	12.5
Salmonella (L3B3)	-	10	10	10	11
Salmonella (L5B1)	8	8	9	10	10
Salmonella (L5X1)	8	8	11	12	12
Salmonella (L5X4)	8	9	11	12	13
Salmonella (FX2)	10	10	11	12	14
Salmonella (HB1)	8	10	12	12	12
Salmonella (L2 B3)	-	-	10	10.5	11
Salmonella (S1B3)	-	8	8	9.5	10
Salmonella (S1X2)	8	9	11.5	11.5	12
Salmonella (S2B1)	8.5	10	11	11	12.5
Salmonella (S2X1)	-	8	9.5	10	10

'–' indicates no zone of inhibition
L1-B2, L3B1, S2X1 etc. are code number of the organisms given during isolation.

Out of the strains tested, most of the organism were resistant to 4 antibiotics; 93.75% were resistant to co-trimaxazole, while 90.62% were resistant to cephotaxime, nalidixic acid and Tetracycline each. However, most of them appeared to be sensitive to other antibiotics such as ceftriaxone (21.87%) Ciprofloxacin (78.125%) and gentamicin (78.125%).

Antimicrobial activity of *T. arjuna* extract:
Thirteen organisms those were found highly resistance against different antibiotics were selected (Table 2) and subjected to sensitivity test against *T. arjuna* bark extract**.**

Resistant organisms were found sensitive to *T. arjuna* extract. Sensitivity increased with the increasing concentration of extract. For salmonella L5X4, the extract showed 8, 9, 11, 12 and 13 mm zones of inhibition against 1,2, 3, 4 and 5 mg/disc of *T. arjuna* extract respectively. Zone of inhibition increased with the increase of concentration.

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DISCUSSION

The aim of this study was to isolate, identify and characterize emerging multi drug resistant organisms, *Salmonella.* Mostly, infection with anti-microbial resistant *Salmonella* is acquired by eating contaminated foods of animal origin (Fey *et al.*, 2000). According to the Infectious Diseases Report released by the World Health Organization (WHO) in 2000, such organisms have become prevalent worldwide (WHO, 2000).
Bacterial species of the family *Enterobacteriaceae*, such as *Salmonella* is one of the most common inhabitants of the intestinal tract of human, domestic and wild animals. *Salmonella* can survive for long periods under refrigeration. Survival in dry environments is a characteristic of these organisms. *Salmonella* are among the major bacterial pathogens of poultry in the world and *Salmonella* infection in humans mostly results from the ingestion of contaminated poultry (Carli *et al.,* 2001).
Poultry is essential to the national economy of Bangladesh and the welfare of human beings as well. Several constraints- the diseases, poor husbandry, low productivity and shortage of food affect the optimal performance of this industry in Bangladesh (Haque *et* al., 1991). Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic diseases of poultry in Bangladesh (Bhattacharjee *et al*., 1996).

In this study, isolated organisms were subjected to antibiotic sensitivity test against 7 different standard antibiotics. Among these four antibiotics i.e. co-trimaxazole, cephotaxime, nalidixic acid and tetracycline were less effective, while. ceftriaxone, ciprofloxacin and gentamicin were more effective. In this study, Ciprofloxacin and gentamicin were found to be the most potent drugs.

Antibiotics are used worldwide both in veterinary and human medicine. Resistance may be natural or acquired. Among the Salmonella obtained from our research 93.75% were resistant to co-trimaxazole, while 90.62% were resistant to cephotaxime, nalidixic acid and Tetracycline each.. However, most of them appeared to be sensitive to large number antibiotics such as ceftriaxone (21.87%), ciprofloxacin (78.125%), gentamicin (78.125%).

Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile (Sani1 et al., 2009). The study of antimicrobial resistance has been a major driving force in the understanding of many genetic and biochemical processes in bacterial cells. In addition, it has direct clinical value in making more effective use and development of antibiotics. *Salmonella* are among those known to carry plasmids, which encode for drug resistance. This implies that widespread use of anti-microbials in animals or humans may cause an increase in the frequency of occurrence of bacterial resistance to other anti-microbial as plasmids may encode resistance to additional anti-microbial agents (Salehi *et al.,* 2005). Most of these antibiotics are added in poultry feed, as supplement and obvious lack of control on the antibiotic usage may be the probable cause for their high resistance against them (Murugkar *et al.,* 2005). After each new antibiotic becomes widely used, antimicrobial resistant genes eventually emerge either by being mobilized from obscure strains or by evolving from ancestral genes.

Though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has developed. Medicinal plants are natural resources, yielding valuable products, which are often used in the treatment of various ailments. Plant materials remain an important resource for combating illnesses, including infectious diseases, and many of the plants have been investigated for novel drugs or templates for the development of new therapeutic agents (Karthy et al., 2009).

Thus, another major interest of this piece of research was to find out antibacterial activity of *T .arjuna* plant extract against resistant organisms. Antimicrobial activity of bark extract of *T. arjuna* against 13 selected isolates of *Salmonella* was determined. *Salmonella* (FX2) did not show any zone of inhibition against six antibiotics. But it showed highest zone of inhibition (14 mm) against *T. arjuna* extract.

Salmonella (L1-B2) was resistant against five antibiotics. It showed 8mm ,10mm, 10mm ,11mm and 12mm zones of inhibition against 1mg/disc, 2mg/disc, 3mg/disc 4mg/disc and 5mg/disc of *T. arjuna* extract, respectively. *Salmonella* (L5-X4) was resistant to Tetracycline, Cephatoxim, Ceftriexone, Cotrimaxazole, and Naloxodicacid. It was found sensitive against *T. arjuna* bark extract. *Salmonella (*H-B1*)* was resistant to five antibiotics but was found to be sensitive against plant extract. *Salmonella* (Lg2-B3) was resistant against four antibiotics. Extract concentrations of 1 mg/disc and 2 mg/disc gave no zone of inhibition but organisms were sensitive to 3 mg/disc, 4 mg/disc and 5 mg/disc. During the course of the anti-microbial screening, it was found that among the 13 selected isolates *Salmonella* (L3-B1), *Salmonella* (L5-X4), *Salmonella* (F-X2), *Salmonella* (S2-B1) showed good sensitivity to crude extract of *T. arjuna*

The present study justifies the claimed uses of *T .arjuna* in the traditional system of medicine to treat various infectious diseases caused by the microbes. This study also encourages cultivation of the highly valuable plant in large-scale to increase the economic status of cultivars in the country. The obtained results may provide a support to use the plant in traditional medicine. Based on this, further chemical and pharmacological investigations to isolate and identify major chemical constituents in *T. arjuna* and to screen other potential bioactivities may be recommended.

It appears from the results that the crude extract was effective against most *Salmonella* isolates. Its potential application in the treatment of bacterial infection in poultry industry of Bangladesh would therefore be promising. This work will lead to innovate the active constituents of the plant *T. arjuna* for the antibacterial activity. On the basis of the result obtained in this present investigation, we conclude that the ethanol extract of *T. arjuna* had significant *in vitro* antimicrobial activity.

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