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Antibiogram and Plasmid Profile Analysis of Isolated Poultry *Salmonella* of Bangladesh

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Abstract: Antibiogram study and plasmid profile analyses were conducted to find out the correlation of the recently isolated *Salmonella* organisms of Bangladesh. Antibiogram study revealed that the isolates were highly sensitive to Ciprofloxacin, Cephalexin and Kanamycin. In the plasmid profile analysis it was observed that out of 4 isolates of Mymensingh district (M_1 to M_4), 3 isolates showed 3 plasmid bands in each and 1 isolate showed 4 plasmid bands. The estimated size of the plasmid bands of M_1 , M_2 , M_3 and M_4 were (33.5, 24.50, 4.0, 3.0); (33.5, 3.0, 1.0); (33.5, 3.0, 1.0) and (5.0, 4.0, 1.8) kb, respectively. On the other hand among the isolates of Feni district (F_1 to F_9), 6 isolates (F_4 , F_5 , F_6 , F_7 , F_8 and F_9) showed 5 number of plasmid bands in each and the estimated plasmid band size were same (33.5, 5.0, 4.0, 2.0, 1.8). The plasmid bands of other 3 isolates (F_1 , F_2 and F_3) were found to be different and the estimated size were recorded as 4 (33.5, 5.0, 1.6, 1.5); 3 (33.5, 1.6, 1.5) and 1 (0.7), respectively. Among the isolates of Dhaka district (D_1 to D_{11}), no plasmid band was found to be detected in 3 isolates (D_2 , D_5 and D_6). The number of plasmid bands of other isolates of D_1 , D_3 , D_4 , D_7 , D_8 , D_9 , D_{10} and D_{11} were 4 and the estimated size were determined as (33.5, 15.0, 2.0, 1.0); 5 (33.5, 15.0, 3.0, 2.0, 1.0); 5 (33.5, 5.0, 4.0, 1.8, 1.3); 3 (33.5, 3.0, 1.0); 3 (33.5, 3.0, 1.0); 1 (33.5); 2 (33.5, 3.0) and 2 (33.5, 4.0) kb, respectively. All these isolates were tested for antibiotic sensitivity against 8 commonly used antibiotics belonging to different groups. From the antibiogram study it was revealed that all the isolates of Mymensingh and Dhaka district were 100% resistant to Cloxacillin and among the isolates of Feni district 100% were resistant to Erythromycin and Cloxacillin. Plasmid profile analysis of the isolated *Salmonella* organisms revealed that the isolates carrying multiple plasmids which might be the cause of various degree of antibiotic resistance.

Key words: Antibiogram, plasmid profile, poultry *Salmonella*

INTRODUCTION

Avian Salmonellosis is an important as both a clinical disease in poultry and as a source of food borne transmission of disease to humans. The incidence of Salmonellosis in Bangladesh was found to be 9.28%^[1] The overall seroprevalence of *Salmonella* infection was found 22.77%^[2]. Village chickens can act as a reservoir of Salmonellosis. The epidemiology of fowl typhoid and pullorum disease caused by *Salmonella gallinarum* and *Salmonella pullorum*, respectively in poultry, particularly with regard to transmission from one generation to the next is known to be closely associated with infected eggs. During recent years, the widespread use of antibiotics in the field of Veterinary medicine have resulted in the development of increasing numbers of bacterial strains possessing resistance to many antibiotics^[3,4]. This was become particularly evident among strains of *Salmonella*. Detailed investigations into the epidemiology of

Salmonellosis revealed that the property of multiple drug resistance could be transferred through conjugation from resistant strains of *Salmonella* to another by means of plasmid, which occur in cytoplasm of the donor bacterium and multiply independently of the chromosomal DNA. Thus, a new bacterium with a resistance factor emerges that is resistant to one or more antimicrobial agents^[5]. So the present study was conducted to find out the correlation between antibiotic sensitivity pattern and plasmid profile of the poultry *Salmonella* isolates of Bangladesh.

MATERIALS AND METHODS

The study was conducted within June 2003 to July 2004.

Cultural media and reagents: Nutrient Broth (NB), Selenite Broth (SB), Luria- Bertani (LB) broth, Blood Agar

(BA), Salmonella-Shigella (SS) agar, Brilliant Green agar (BGA) and McConkey agar were used for culture and isolation of *Salmonella*. Ethidium bromide, phenol, ethanol, isopropanol, agarose gel, chloroform, TE-buffer, RNAs, tracking dye, reference marker supermix DNA ladder which contains 13 bands of double stranded linear DNA fragments ranging from 500 bp to 33.5 kb which was purchased from Bangalore Genei Pvt. Ltd., India were used for the plasmid DNA extraction and Agarose gel electrophoresis.

Determination of antimicrobial sensitivity pattern: Sensitivity of isolates of *Salmonella* to different antimicrobial agents was performed to determine the drug sensitivity pattern and to interpret the results (Table 1). This method allows for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from different diffusion of the antibiotics into the medium surrounding the disc.

Salmonella organisms were grown overnight in NB. The overnight cultured organism was poured on BA media and spread uniformly. Antibiotic discs which were purchased from Mast Group Ltd. Merseyside, UK were applied aseptically to the surface of the inoculated plates at an appropriate arrangement with the help of sterile forceps and incubated at 37°C for 24 h^[6].

Plasmid DNA extraction: Smaller circular plasmid DNA molecules were extracted from the huge chromosomal DNA for analysis of the DNA.

Plasmid extraction procedure: The selected bacterial strain (single colony) was grown overnight in LB broth at 37°C with aeration using the orbital shaker. 1.5 mL overnight culture was taken in eppendorf for plasmid DNA extraction. The plasmid DNA from *Salmonella* isolates was extracted through Mini alkaline lysis by SDS^[7].

Agarose gel electrophoresis of plasmid DNA: Electrophoresis was carried out in a horizontal gel apparatus. The method followed for agarose gel electrophoresis was as described by Maniatis *et al.*^[8].

Table 1: Antimicrobial agents and their disc concentration

Antimicrobial agent	Disc concentration	Antimicrobial agent	Disc concentration
Erythromycin	15 µg	Kanamycin	30 µg
Cloxacillin	10 µg	Cephalexin	30 µg
Ampicillin	10 µg	Ciprofloxacin	5 µg
Nalidixic acid	30 µg	Chloramphenicol	30 µg

RESULTS AND DISCUSSION

Antibiogram study of *Salmonella* organisms:

Twenty-four *Salmonella* isolates from Mymensingh (M₁ to M₄), Feni (F₁ to F₉) and Dhaka district (D₁ to D₁₁) was tested for antibiotic sensitivity against 8 commonly used antibiotics belonging to different groups. From the antibiogram study it was revealed that all the isolates of Mymensingh district were 100% resistant to Cloxacillin. 50% of the isolates were resistant and 50% were less sensitive to Erythromycin. Twenty five percent of the isolates were resistant, 25% were less sensitive and 50% were moderately sensitive to Ampicillin and Nalidixic acid. Seventy percent of the isolates were moderately sensitive and 25% were highly sensitive to Kanamycin and Cephalexin. Fifty percent of the isolates were moderately sensitive and 50% were highly sensitive to Ciprofloxacin. Fifty percent of the isolates were less sensitive and 50% were moderately sensitive to Chloramphenicol (Table 2 and Fig. 1).

Among the isolates of Feni district 100% were resistant to Erythromycin and Cloxacillin. About thirty three percent of the isolates were resistant and 66.66% were moderately sensitive to Ampicillin. About forty four percent the isolates were resistant, 22.22% were less sensitive and 33.33% were moderately sensitive to Nalidixic acid. Hundred percent of the isolates were moderately sensitive to Kanamycin. 33.33% were less sensitive and 66.66% were moderately sensitive to Cephalexin. About 60% of the isolates were less sensitive and 33.33% were highly sensitive to Ciprofloxacin. About 60% of the isolates were less sensitive and 33.33% were moderately sensitive to Chloramphenicol (Table 2 and Fig. 2).

Among the isolates of Dhaka district, 100% of the isolates were resistant to Cloxacillin. About 72% of the isolates were resistant and 27.27% were less sensitive to

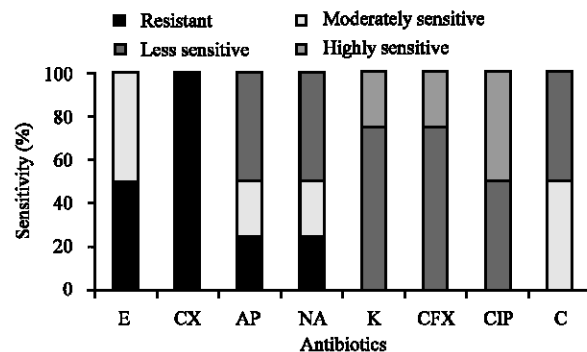


Fig. 1: Antibiotic sensitivity patterns of *Salmonella* isolates of Mymensingh district

Table 2: Antibiotic sensitivity pattern of *Salmonella* organisms isolated from Mymensingh district (M₁ to M₄), Feni district (F₁ to F₉) and Dhaka district (D₁ to D₁₁)

Isolates	Resistance (-)	Less sensitive (+)	Moderately sensitive (++)	Highly sensitive (+++)
M ₁	AP, CX	E, C	NA, CIP	K, CFX
M ₂	CX	E, CX, NA	K, CFX, C	CIP
M ₃	E, CX	-	AP, NA, K, CFX, C	CIP
M ₄	E, CX, NA	C	AP, K, CFX, CIP	-
F ₁	E, CX, NA	CFX, CIP, C	AP, K	-
F ₂	E, CX, NA, AP	CFX, CIP, C	K	-
F ₃	E, CX, AP	-	NA, K, CFX, C, CIP	-
F ₄	E, CX, AP	C	NA, K, CFX	CIP
F ₅	E, CX	-	AP, NA, K, CFX, C	CIP
F ₆	E, CX, NA	CIP, C	AP, K, CFX	-
F ₇	E, CX, NA	CIP, C	AP, K, CFX	-
F ₈	E, CX	NA, CIP	AP, K, C, CFX	-
F ₉	E, CX	NA, CFX, CIP, C	AP, K	-
D ₁	CX, AP	E, C	NA, CFX	K, CIP
D ₂	E, CX, NA	-	K, CIP	AP, CFX, C
D ₃	E, CX, NA	-	K, CFX, CIP	AP, C
D ₄	E, CX, NA	-	AP, K, CFX, CIP, C	-
D ₅	E, CX, NA	K	AP, CFX, CIP	C
D ₆	E, CX, C	NA	AP, K, CIP	CFX
D ₇	CX, AP	E, NA	K, CFX, C	CIP
D ₈	E, CX, NA	-	K, CFX, CIP	C, AP
D ₉	CX, AP	E, NA	K, CFX, C	CIP
D ₁₀	E, CX, NA	-	AP, CIP, C	K, CFX
D ₁₁	E, CX, NA	-	AP, K, CFX, CIP	C

Erythromycin = E, Cloxacilin = CX, Ampicillin = AP, Nalidixic acid = NA, Kanamycin = K, Cephalixin = CFX, Ciprofloxacin = CIP, Chloramphenicol = C and Resistant = -

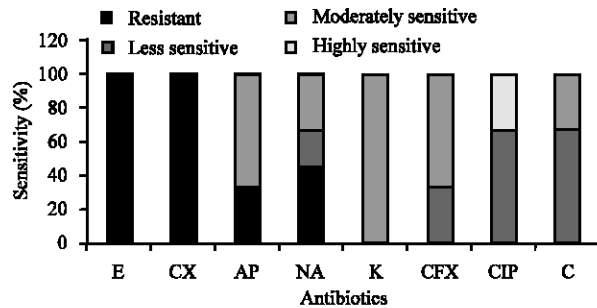


Fig. 2: Antibiotic sensitivity patterns of *Salmonella* isolates of Feni district

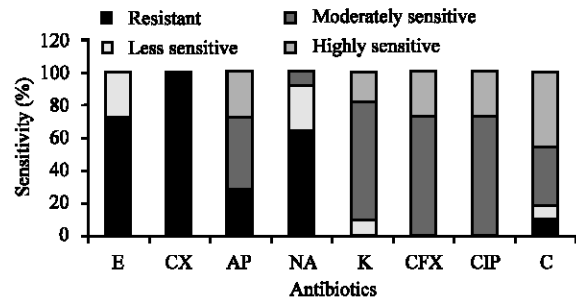


Fig. 3: Antibiotic sensitivity patterns of *Salmonella* isolates of Dhaka district

Erythromycin. About 63% of the isolates were resistant, 27.27% were less sensitive and 9.09% were moderately sensitive to Nalidixic acid. About 9% of the isolates were less sensitive, 72.72% were moderately sensitive and 18.18% were highly sensitive to Kanamycin. About 72% of the isolates were moderately sensitive and 27.27% were highly sensitive to Cephalixin and Ciprofloxacin (Table 2 and Fig. 3).

Plasmid profile of isolated *Salmonella* organism: Plasmid from 24 *Salmonella* isolates were extracted according to the procedure described in materials and methods and analysed by agarose gel electrophoresis. Gel electrophoresis showed a total of 76 different plasmid bands occurring in various combinations

producing all together 13 different plasmid patterns. In the plasmid profile it was observed that out of 4 isolates (M₁ to M₄) of Mymensingh district, 3 isolates contain 3 plasmid bands in each and 1 isolate contain 4 plasmid bands. The estimated size of the plasmid bands of M₁, M₂, M₃ and M₄ were (33.5, 24.50, 4.0, 3.0); (33.5, 3.0, 1.0); (33.5, 3.0, 1.0) and (5.0, 4.0, 1.8) kb, respectively (Table 2 and Fig. 4).

Among the isolates of Feni district (F₁ to F₉), 6 isolates (F₄, F₅, F₆, F₇, F₈ and F₉) contain 5 number of plasmid bands each and which were estimated as same size (33.5, 5.0, 4.0, 2.0, 1.8) and the plasmid bands of another 3 isolates (F₁, F₂ and F₃) were 4 and the estimated size were recorded as (33.5, 5.0, 1.6, 1.5); 3 (33.5, 1.6, 1.5) and 1 (0.7), respectively (Table 2 and Fig. 5).

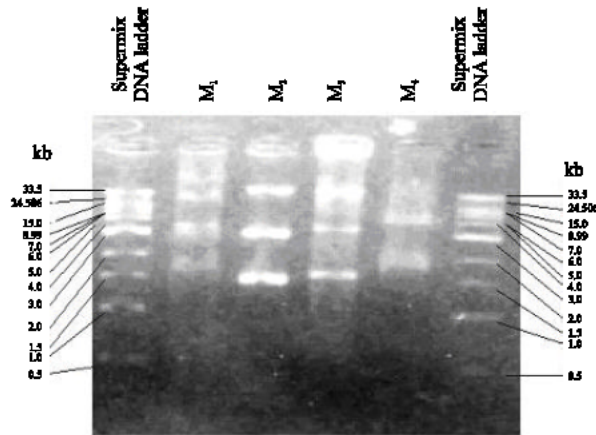


Fig. 4: Plasmid profile of *Salmonella* isolates of Mymensingh district (M₁ to M₄) analyzed by 0.8% Agarose gel electrophoresis after staining with Ethidium bromide and DNA bands were visualized by UV-Transilluminator

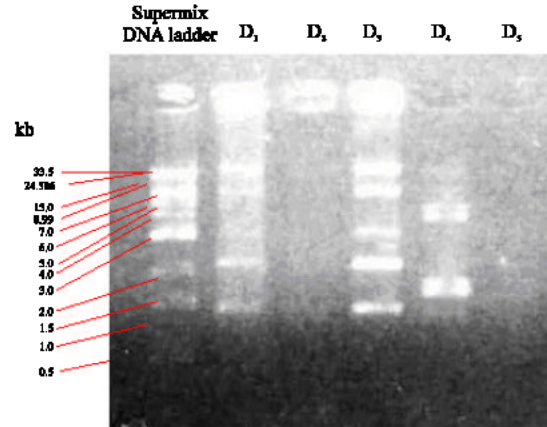


Fig. 6: Plasmid profile of *Salmonella* isolates of Dhaka district (D₁ to D₅) analyzed by 0.8% Agarose gel electrophoresis after staining with Ethidium bromide and DNA bands were visualized by UV-Transilluminator

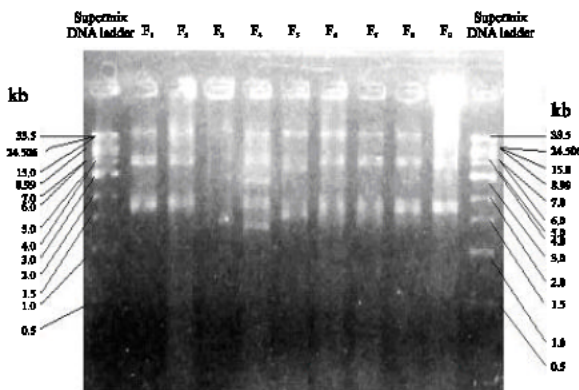


Fig. 5: Plasmid profile of *Salmonella* isolates of Feni district (F₁ to F₉) analyzed by 0.8% gel electrophoresis after staining with ethidium bromide and DNA bands were visualized by UV-Transilluminator

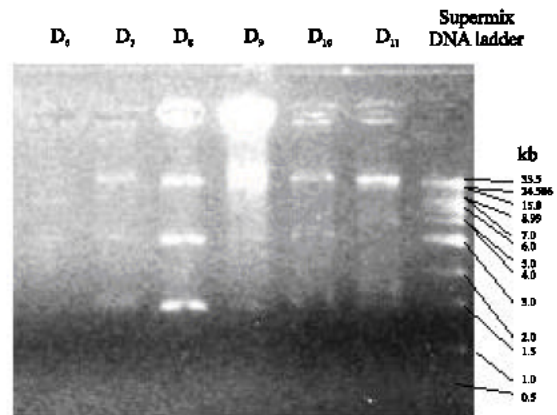


Fig. 7: Plasmid profile of *Salmonella* isolates of Dhaka district (D₆ to D₁₁) analyzed by 0.8% Agarose gel electrophoresis after staining with Ethidium bromide and DNA bands were visualized by UV-Transilluminator

Among the isolates of Dhaka district (D₁ to D₁₁), no plasmid bands were found in 3 isolates (D₂, D₅ and D₆). The number of plasmid bands of other isolates of D₁, D₃, D₄, D₇, D₈, D₉, D₁₀ and D₁₁ were 4 and the estimated size were determined as (33.5, 15.0, 2.0, 1.0); 5 (33.5, 15.0, 3.0, 2.0, 1.0); 5 (33.5, 5.0, 4.0, 1.8, 1.3); 3 (33.5, 3.0, 1.0); 3 (33.5, 3.0, 1.0); 1(33.5); 2 (33.5, 3.0) and 2 (33.5, 4.0) kb, respectively (Table 2, Fig. 6 and 7).

No remarkable correlation between plasmid patterns and drug sensitivity patterns were found. *Salmonella* isolates D₂, D₅ and D₆ contain no apparent plasmid band but these three isolates were resistant to three antibiotics. All other *Salmonella* isolates contain single

or multiple plasmid bands and were resistant to multiple antibiotics showed multiple drug resistance patterns. The plasmid profiles were compared with reference marker, supermix DNA ladder, which contained 13 bands of double stranded linear DNA fragments ranging from 500 bp to 33.5 kb.

The isolated *Salmonella* organisms were highly sensitive to Kanamycin, Ciprofloxacin and Cephalixin, moderately sensitive to Ampicillin, Nalidixic acid and Chloramphenicol and less sensitive to Erythromycin. The isolated *Salmonella* organisms were completely resistant to Cloxacillin. The resistance might be due to routine indiscriminate use of these antibacterial agents in those

Table 3: Plasmid profile and antibiotic resistance patterns of the *Salmonella* isolates

Isolates	Resistance to antibiotics	No. of plasmid bands	Size of plasmid in kb
M ₁	AP, CX	4	33.5, 24.506, 4.0, 3.0
M ₂	CX	3	33.6, 3.0, 1.0
M ₃	E, CX	3	35.5, 3.0, 1.0
M ₄	E, CX, NA	3	5.0, 4.0, 1.8
F ₁	E, CX, NA	4	33.5, 5.0, 1.6, 1.5
F ₂	E, CX, NA	3	33.5, 1.6, 1.5
F ₃	E, CX, AP	1	0.7,
F ₄	E, CX, AP, C	5	33.5, 4.0, 2.0, 1.5, 0.5
F ₅	E, CX,	5	33.5, 5.0, 4.0, 2.0, 1.8
F ₆	E, CX, NA	5	33.5, 5.0, 4.0, 2.0, 1.8
F ₇	E, CX, NA	5	33.5, 5.0, 4.0, 2.0, 1.8
F ₈	E, CX	5	33.5, 5.0, 4.0, 2.0, 1.8
F ₉	E, CX	5	33.5, 5.0, 4.0, 2.0, 1.8
D ₁	CX, AP	4	33.5, 15.0, 2.0, 1.0
D ₂	E, CX, NA	0	-
D ₃	E, CX, NA	5	33.5, 15.0, 3.0, 2.0, 1.0
D ₄	E, CX, NA	5	33.5, 5.0, 4.0, 1.8, 1.3
D ₅	E, CX, NA	0	-
D ₆	E, CX, C	0	-
D ₇	CX, AP	3	33.5, 3.0, 1.0
D ₈	E, CX, NA	3	33.5, 3.0, 1.0
D ₉	CX, AP	1	33.5
D ₁₀	E, CX, NA	2	33.5, 3.0
D ₁₁	E, CX, NA	2	33.5, 4.0

Erythromycin = E; Cloxacillin = CX; Ampicillin = AP; Nalidixic acid = NA; Kanamycin = K; Cephalexin = CFX; Ciprofloxacin = CIP; Chloramphenicol = C; Dhaka = D; Feni = F; Mymensingh = M and kb= Kilobase

selected farms and/or rapid chromosomal mutation and specific plasmid DNA (Table 3). Besides, drug resistance also vary with the presence of multiple or large plasmids in a serovar^[3,4].

The *Salmonella* isolates of three districts were subjected to antibiotic sensitivity test where the isolates of Mymensingh district showed fully resistant to Cloxacillin and Erythromycin and slightly resistant to Ampicillin and Nalidixic acid which is closely related with the results of^[9]. On the other hand, the isolates were fully sensitive to Kanamycin, Cephalexin, Ciprofloxacin and Chloramphenicol. The isolates of Feni district were fully resistant to Erythromycin and Cloxacillin, which was also found^[10]. The isolates of Feni were found fully sensitive to Cephalexin and Ciprofloxacin. The organisms isolated from Feni were found sensitive to Kanamycin and Nalidixic acid. The isolates of Dhaka district showed fully resistance to Cloxacillin and moderately sensitive to Nalidixic acid and Ampicillin. The isolates were highly sensitive to Kanamycin, Cephalexin and Ciprofloxacin.

From the plasmid profile analysis it was revealed that the isolates of Mymensingh and Feni district carried multiple plasmids. The isolates of Dhaka district carrying no plasmid in three isolates, single plasmid was found in one isolate and the rests of the isolates contain multiple plasmids which correlates with the results of Lee *et al.*^[11] and Rehman^[12].

In the present study no remarkable correlation between plasmid patterns and drug sensitivity patterns were found. *Salmonella* isolates D₂, D₅ and D₆ contain no apparent plasmid band but these three isolates were also resistant to Erythromycin, Cloxacillin and Nalidixic acid. All other *Salmonella* isolates contained single or multiple plasmid bands and showed multiple drug resistance patterns. These multiple drug resistance patterns of the *Salmonella* isolates of this study might be due to drug resistance genes carried out by the different plasmids bands^[13].

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