

Antibiotics effect on *salmonella spp* .isolated from young chicks in chicken fields

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Abstract: The aim of the present study was the isolation and diagnosis of the bacteria that cause bacillary white diarrhea from young chicks, identification the present percentage, and establishment the antibiotic effect on bacteria. Bacillary white diarrhea disease is a major health problem as the causative agent *Salmonella pullorum* has developed resistance to many antimicrobial agents. We studied 88 samples feces collect from chicken in the chicken field in Hilla city (Babylon province.). These samples were cultured on selective and differential media; biochemical tests and antimicrobial effect on the types of these bacteria was studied. Antimicrobial susceptibility testing was done by disc diffusion method. The results showed that, 68 isolate were *S. pullorum* and 20 isolate were *S. enteritidis*. Regarding Antibiotics sensitivity test, *S. pullorum* and *S. enteritidis* were highly sensitive to ciprofloxacin, (100%), (90%) respectively, followed by amikacin (88%) for *S. pullorum* and (80%) for *S. enteritidis*. While erythromycin, enrofloxacin and chloramphenicol were not effective against tested isolates. The findings of the present study revealed the presence of multidrug resistant *Salmonella* species in young chicks from chicken fields in Hilla city.

Keywords: Antimicrobial susceptibility, *Salmonella* species, Bacillary white diarrhea disease, Young Chicks.

I. Introduction

Salmonella infection is one of the most important global poultry diseases which are caused by different *Salmonella* species (1, 2). More than 2,500 serotypes have been described mostly under the species (3,4). Salmonellosis, especially the one that manifests as Pullorum disease, is one of the commonest diseases of local poultry, which causes severe economic loss to the industry. *S. Pullorum* and *S. enteritidis* are both normally reported to attack poultry (5, 6). Few diseases are more devastating to poultry producers than pullorum. In 1899, *Salmonella pullorum* was the first identified bacteria that causes heavy losses in chicks and poults and decreases the productivity of adult birds.

An infection that begins with a single bird can spread quickly through a flock, causing mortality as high as 80 % or more (7, 8). Pullorum disease is usually transmitted in the egg hen, but may also be spread through breathing or consuming contaminated dust, down, droppings or broken eggs, mating with an infected male. Infections with *Salmonella pullorum* can result in acute systemic disease and a high incidence of mortality in young poultry. Vertical transmission of *S. pullorum* from infected parent flocks, magnified by horizontal transmission in the hatcher, can cause economically devastating losses among chicks or poults (9, 10). The use of antimicrobials in veterinary medicine as food animal growth promoting agents during the past decade has created enormous pressure for selection of antimicrobial resistance among bacterial pathogens worldwide (11,12,13). Nowadays, there is increasing concern about the development of multidrug resistance in bacteria causing zoonosis and having an important animal reservoir, such as *Salmonella* strains (14, 15). The aim of the present study was the isolation and diagnosis of the bacteria that cause bacillary white diarrhea from young chicks, identification the present percentage, and establishment the antibiotic effect on bacteria.

II. Material And Methods

1. Sample collection:

Fecal samples were collected from chicken fields in Hilla city (during a period from February to August 2013). A total of 88 specimens of *Salmonella* were collected, all the specimens were isolated and identified culturing and biochemically using standard procedures. Subcultures were done on nutrient agar, MacConkey agar, *Salmonella-Shigella* agar (SS) and Xylose lysine deoxycholate agar (XLD) and incubated at 37°C for 18 to 24 hr. All the isolates were stored in brain heart infusion broth with 15% glycerol at -20°C until further use (10, 16, and 17).

2. Antibiotic susceptibility testing:

Antimicrobial susceptibility testing of the isolates to various routinely used antibiotics was determined by disc diffusion technique on Muller Hinton agar using commercially available discs following CLSI guidelines (2010). The panel of antimicrobials was show in Table (1). Sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay

for 3-5 minutes. Sterile forceps was used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plate was incubated at 37°C for 18-24 hours. By using Meter rule on the underside of plate, the diameter of each zone of inhibition was measured in millimeter. Zone diameter for isolate was compared with CLSI Published Limits; interpretative chart was then used to interpret the zone sizes of inhibition. Result was recorded as susceptible, intermediate susceptible, or resistant based on the Zones sizes of each antimicrobial disc used. The results then interpreted according to CLSI documentation (18).

Table (1): Interpretive standards for disc diffusion susceptibility testing of salmonella (18).

Antibiotic disc	Symbol	Disc concentration	Diameter of zone of inhibition (mm)		
			Susceptible	intermediate	Resistant
Amikacin	AK	30µg	≥17	15~16	≤14
Chloramphenicol	C	30 µg	≥18	13~17	≤12
Rifampin	RA	5 µg	>20	17~19	<16
Ciprofloxacin	CIP	5 µg	≥21	16~20	≤15
Enrofloxacin	ENR	5 µg	≥20	17~19	≤16
Gentamicin	CN	10 µg	≥15	13~14	≤12
Erythromycin	E	15 µg	≥23	14~22	≤13

III. Results And Discussion

1. Isolation of bacteria :

The results were shown in Fig. (1) indicated that growth was observed in 68(77.27 %) *Salmonella pullorum*, while 20(22.73%) *Salmonella enteritidis* from 88 total specimens.

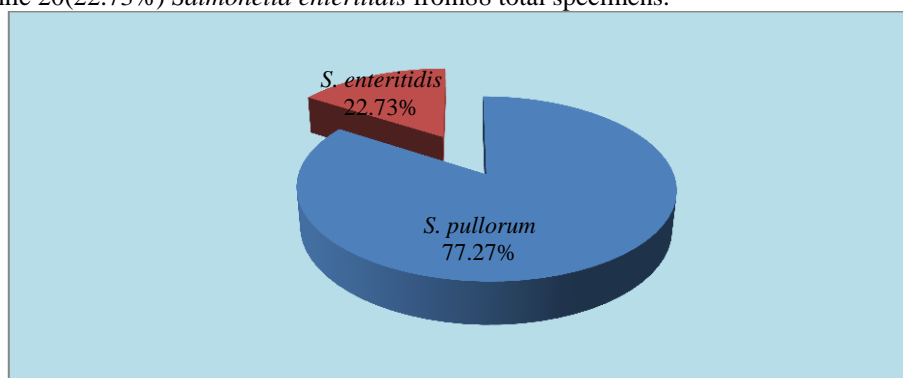


Figure (1): Percentage of isolations.

2. Laboratory identification of salmonella spp.:

In this study, salmonella spp. isolates were identified by investigation of colonial morphology on MacConkey agar in which the colonies appeared smooth, colorless and gram negative non-sporogenic, rod shape. While growth on Xylose lysine deoxycholate agar (XLD) and Salmonella – shigella agar (SS) as small red translucent colonies without black color in center of colonies and motile as *Salmonella pullorum*, while *Salmonella enteritidis* has been non-motile and growth on XLD and SS agar may have a central black spot due to H₂S production (19, 20). According to the age (from 1 to 12 days), chick samples distribution showed high infection rate with *Salmonella pullorum* in small ages chicks, while *Salmonella enteritidis* infection was high in large chicks as shown in Table (2).

Table (2): The distribution of isolates is according to the age of chick.

No.	Chick samples			
	Age (days)	Isolate		Total (%)
		<i>S. Pullorum</i>	<i>S. enteritidis</i>	
1	5	36	0	36(41)
2	7	24	8	32 (36.3)
3	12	8	12	20(22.7)
Total		68	20	88(100)

Many studies have been conducted by many researchers in different parts of the world establishing the significance of pullorum disease in chicks. The above two species of *Salmonella* spp. were encountered in this study. Among the *Salmonella* spp. isolated *S. pullorum* was predominant, followed by *S. enteritidis*. In the present study, specific enriched media and biochemical tests were used for the isolation and identification of *Salmonella* spp. which was also used by a number of researchers (2, 8, 20). The colony characteristics of *Salmonella* spp. exhibited colorless, smooth, transparent on MacConkey agar; black or colorless on SS agar and translucent, opaque, smooth colonies on nutrient agar were similar to the findings of other authors (1, 10, 21). In indole test, all the test isolates (n = 88) did not develop any red color which indicated the *Salmonella* isolates were negative to indole test (22). In case of reaction in TSI agar slant, 77.27% of the isolates (n =68) produced red slant, yellow but without the production of H₂S and no gas, while 22.73 % isolates (n =20) were associated with the production of H₂S which strongly supported the observations of others (8, 10).

3. The sensitivity of salmonella spp to the antibiotic by using disk diffusion test:

In this test, 88 samples were included and the antimicrobial susceptibility patterns of *Salmonella* spp. against various antibiotics were studied. Out of 68 *Salmonella pullorum* isolates, 68(100%) were resistant to erythromycin, enrofloxacin and chloramphenicol. On the other hand, 68 (100%) were susceptible to ciprofloxacin, 60(88%) were susceptible to amikacin, 52(76%) were susceptible to rifampin and 45(66%) were susceptible to gentamicin as shown in Fig. (2).

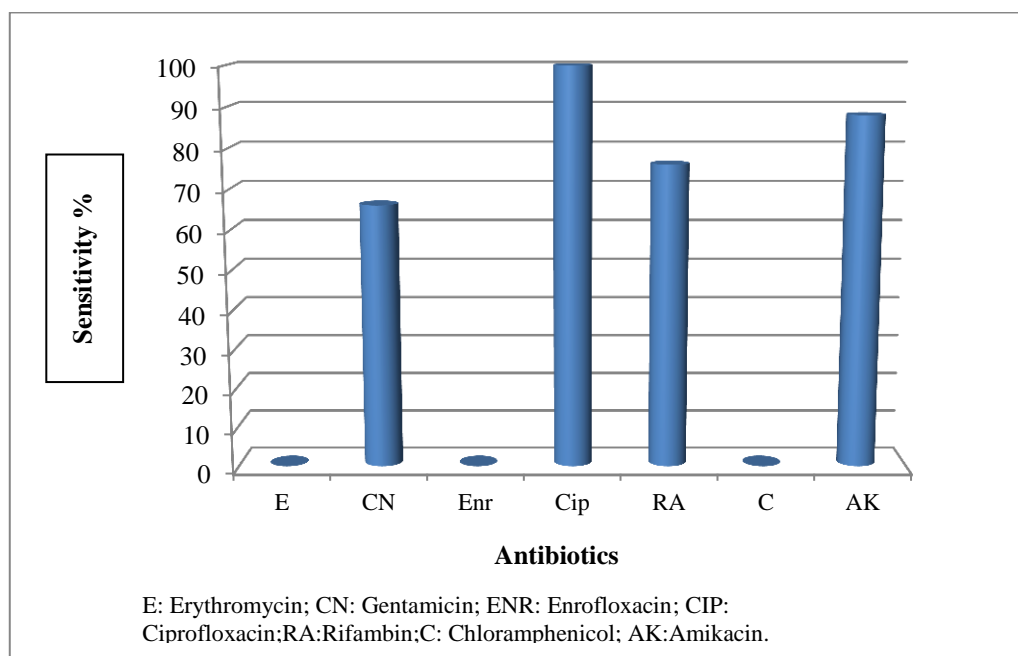


Figure (2): Sensitivity rate of *S. pullorum* to different antibiotics.

Out of 20 *Salmonella enteritidis* isolates, 20(100%) were resistant to erythromycin, enrofloxacin, chloramphenicol and gentamicin. Furthermore, 18 (90%) were susceptible to ciprofloxacin, and 16(80%) were susceptible to amikacin Fig. (3). In antimicrobial susceptibility testing, out of 68 *Salmonella pullorum* isolates, 68(100%) were resistant to erythromycin, enrofloxacin and chloramphenicol. Out of 20 *S. enteritidis* isolate, 20(100%) were resistant to erythromycin, enrofloxacin, chloramphenicol, and gentamicin. The antibiotic resistance of *Salmonella* strains of avian origin was attributed to chromosomal mutation, gene transfer mechanisms like conjugation, transduction and transformation (12, 23, 24, 25 and 26). Additionally, out of 68 *Salmonella pullorum* isolates, 68(100%) were susceptible to ciprofloxacin, 60(88%) were susceptible to amikacin, 52(76%) were susceptible to rifampin and 45(66%) were susceptible to gentamicin, while 20 *S. enteritidis* isolate, 18 (90%) were susceptible to ciprofloxacin, and 16(80%) were susceptible to amikacin. These findings are also very close to many studies (15, 27, 28 and 29). In particular the long term use of insufficient doses is regarded as one of the major factor responsible for development of antibiotic resistance (30).

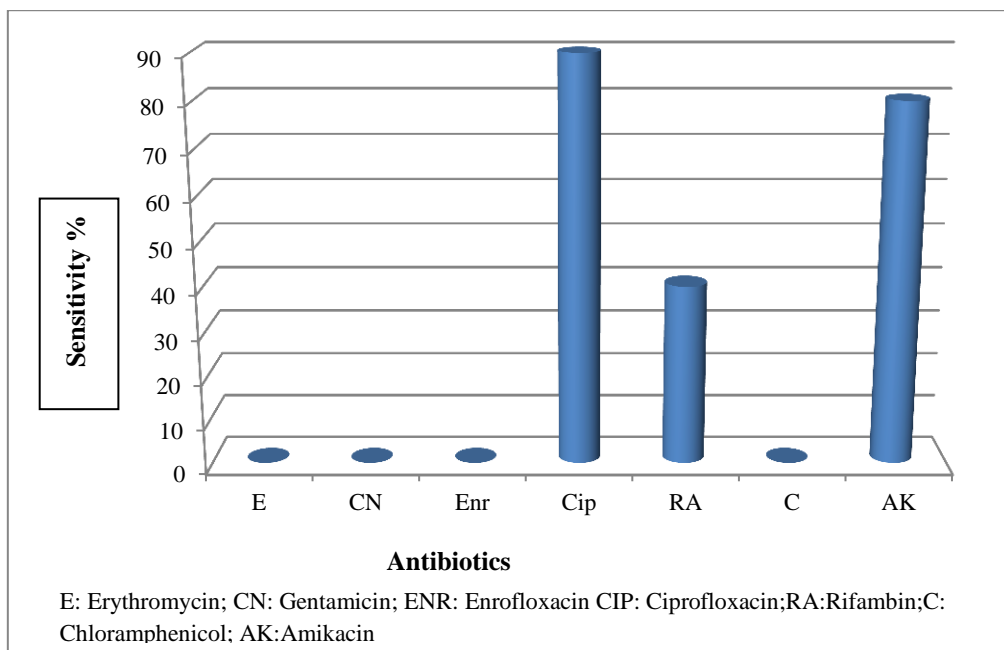


Figure (3): Sensitivity rate of *S. enteritidis* to different antibiotics.

IV. Conclusion

In this study we conclude that rate of Bacillary white diarrhea infection in young chicks was high. *S. pullorum* was the predominant isolate. This study revealed that our isolates showed good sensitivity to most antimicrobials agents tested. The increasing prevalence of erythromycin, enrofloxacin and chloramphenicol resistance associated with reduced susceptibility to ciprofloxacin suggested that ciprofloxacin treatment may not be effective for serious Salmonella infection.

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