

RESEARCH ARTICLE

AN ANTIBIOGRAM STUDY OF SALMONELLA AND E. COLI ISOLATED FROM BROILERS FARMS IN AL GUBA AND SHAHAT.

Abdulkarim F. H. Adam¹, Tufahah .M .O. Atiyahullah¹, Najwa. A. Ismaael¹,Osama K. Gaidan² Tawfig. El Tigani.Mohamed¹ and Atiyah. A. A. salih³.

- 1. Department of preventive medicine and public health Faculty of veterinary medicine. Omar ALmukhtar University, P.O.Box 919 ELBeida, Libya.
- Department of Animal medicine and Surgery Faculty of veterinary medicine. Omar ALmukhtar 2. University, P.O.Box 919 ELBeida, Libya.

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Abstract

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Keywords:-

Salmonella, E.coli, Antimicrobial resistance, Broilers.

In the present study, the isolation of salmonella and E.coli and their antibiogram patterns in broiler farms in Alguba and Shahat were performed. Tissue samples including (liver, heart blood, spleen, exudates air sac, and intestine) were collected from 60 fresh dead or sick chickens obtained from five and three broiler farms located in Shahat and Alguba, respectively. The isolation and the identification Salmonella and E.coli were performed according to standard methods and the resistance to 13 antimicrobial agents was determined by using Kirby-Bauer disk diffusion method. In the results, E.coli and Salmonella were isolated at percentage of 21.6% and 11.6% respectively. Resistance pattern of Salmonella in descending order was respectively Amoxicillin (100%), Ampicillin(100%), Erythromycin (100%), Doxycycline (50%), Oxytetracyclin (50%), Tetracycline (50%), and Spectinomycin (25%). Whereas E.coli isolates were 100% resistant to Amoxicillin, Ampicillin, Tetracycline, Erythromycin, followed by Enrofloxacin (80%), Oxytetracyclin (80%), Streptomycin (80%), Doxycycline (80%), Sulphamethoxazole (40%), Spectinomycin (60%), and Neomycin (60%). Also in this study, all of salmonella isolates were sensitive to Gentamycin or Colistin sulphate. sensitive pattern of E.coli was recorded to Colistin, followed by Sulphamethoxazole, and Spectinomycin. Moreover, all the isolates of Salmonella and E.coli showed multiple antimicrobial resistances. It is concluded that the rational use of antibiotics broilers farming system should be applied in order to prevent the emergence of antimicrobial resistance pathogens. Additionally, Colistin could be the drug of choice to treat the infection with Salmonella and E.coli.

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Introduction:-

E.coli and Salmonella have been reported to be the common causes of infectious diseases at all ages of birds (Hassan et al, 2014). Escherichia coli are the most prevalent facultative anaerobic species which normally inhibit

Corresponding Author:- Abdulkarim F. H. Adam.

Address:- Department of preventive medicine and public health Faculty of veterinary medicine. Omar ALmukhtar University, P.O.Box 919 ELBeida, Libya.

the gastrointestinal tract of both human and animals. Usually it is a harmless microbe, but under immune suppressive condition, it can cause a variety of illnesses (Friedman et *al*, 2002). *E. coli* are the causative agent of airsacculitis, pericarditis, septicemia, and death, which are of direct contact with economic significance (Hofstad et *al.*, 1984).

Salmonella have been isolated from nearly all vertebrates, and the infections with *Salmonella* have been recorded in both animal and human worldwide. In fact, *Salmonella* are responsible for pullorum diseases, fowl typhoid, and fowl paratyphoid in poultry (**Hassan et al, 2014**).

The growing increase in antimicrobial resistance pathogens such as Escherichia coli and Salmonella has become a critical public health concern (Hanson et al, 2002). In other word, poultry meat ranks first or second in food -related illness (Sams AR 2001). Processed raw poultry meat naturally harbors many types of bacteria and most of these bacteria are pathogenic to humans (Mead GC 2001).

The contamination can occur at several points throughout the processing operation (Ayres CP 1995). This may lead to the transfer of the resistant genes located in Salmonella and other Zoonotic bacteria from food animals to humans through consumption of contaminated food and food products (Molla et al, 2003)

Additionally, a considerable number of antimicrobials, which commonly used in the treatment of Salmonella and other bacterial infections in veterinary field, are also practiced for humans (Gay et al, 1994). This may limit the therapeutic possibilities in the treatment of diseases caused by these pathogens in humans and domestic animals in general, and poultry in particular (Nicole et al., 2000, Akbar and Anal, 2013).

Due to the indiscriminate use of antibiotics in the poultry farm and the emergence of drug resistant nonpathogenic E. coli from animal origin, which could harbor the human intestine (**Hassan et al, 2014**), this study was carried to determine the in vitro sensitivity of Salmonella and E.coli isolated from broilers to different antimicrobial agents in Alguba and Shahat farms.

Material and Methods:-

Questionnaire:-

A total of 15 questions structured were managed by the research team and administered to five and three broiler farms in Shahat and Alguba respectively. The objective of this questionnaire was to determine the most commonly used antimicrobial in broiler farms .

Isolation and Identification:-

This study was conducted on 60 fresh dead or sick chickens collected randomly from five and three broiler farms in Shahat and Alguba respectively, located in Al Jabal Alakhdar Region during the period of January to February.

Samples were aseptically collected from internal organs including liver, heart blood, spleen, exudates air sac and processed according to (**Quinn** *et al*, **2002**). All samples were pooled, and the isolation of *Salmonella* and *E.coli* was performed by using the method described by (**Quinn** *et al*, **2002**; **Abd El Tawab** *et al*, **2015**) on MacConkey's agar (Oxoid), Eosin Methylene blue agar (Oxoid), Briliant green agar (Oxoid), Salmonella – Shigella agar (Oxoid), and Xylose lysine desoxycholate agar (Oxoid) (**Hanson** *et al*, **2002**; **Molla** *et al*, **2003**). Series of biochemical tests, including Indole reaction (Oxoid), Methyl red test (Oxoid), Voges Proskauer test (Oxoid), Citrate utilization test (Himedia), Triple sugar iron agar test (Oxoid), Urease test (Oxoid), H2S production test (Oxoid), and Motility test (Oxoid) were used for the confirmation of suspected isolates (**Quinn** *et al*, **2002**; **Cappuccino and Sherman, 2008**).

In Vitro an antibiogram study:-

All isolates were tested in vitro for their sensitivity and resistance to different type of antibiotics according to Kirby-Bauer Disk Diffusion method (**Bauer** *et al*, **1996**; **Soomro** *et al*, **2009**). Where, briefly, all isolates were compared with 0.5 Mc Farland turbidity standards to obtain 1×10^8 CFU/ ml. Then the isolates were inoculated on Mueller Hinton agar (Oxoid), and kept for 5 min at room temperature. Afterward, the antibiotics disks were placed on the inoculated plates and incubated aerobically for 24 h at 37 C°. Antibiotics disks of Oxoid used in this study were Amoxicillin (10 µg), Neomycin (30µg), Enrofloxacin (5µg), Sulphamethoxazole (25µg), Doxycycline (30 µg), Erythromycin (15 µg), Colistin sulphate (25µg), Oxytetracyclin (30µg), Spectinomycin (100µg), Gentamycin $(10\mu g)$, Ampicillin $(10\mu g)$, Streptomycin $(10\mu g)$, and Tetracycline $(30\mu g)$. The antimicrobial agents were categorized into susceptible, intermediate, and resistant categories according to (NCCLS., 2007).

Results:-

The questionnaire results:-

The most commonly used antimicrobial agents were Enrofloxacin (100%) followed by diclazuril (33%) in Alguba farms. In terms of Shahat farms, it was reported that the most commonly used antibiotics were diclazuril (100%), followed by Enrofloxacin (80%) and Colistin (20%) **Figure 1**.

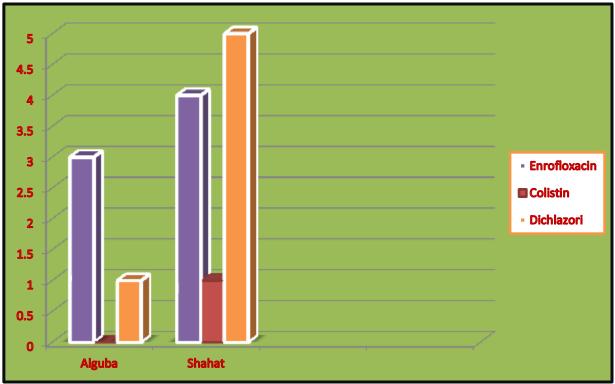


Figure 1:-The most common used antibiotics

Bacteriological examination:-

Under the condition of this study, out of 60 birds, *E. coli* was isolated from 13 birds (21.6%) and *Salmonella* from just 7 broilers (11.6%)

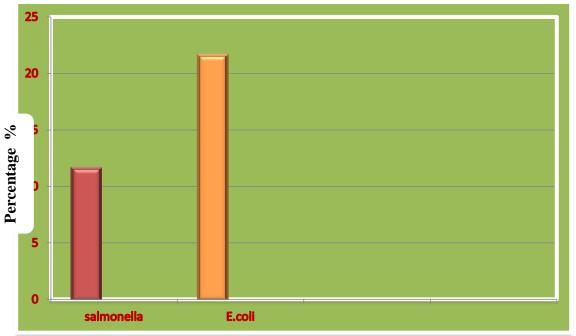


Figure 2:-Percentage of Salmonella and E.coli isolated from broiler Sensitivity test

Sensitivity test:-

In the antibiogram study, all *Salmonella* isolates (100%) were found to be highly resistant to Amoxicillin and Ampicillin, followed by Erythromycin (75%). Two of *Salmonella* isolated were (50%) resistant to Doxycycline, Oxytetracyclin, and Tetracycline. Less resistance of isolates (25%) was found against Enrofloxacin and Spectinomycin. (75%) of isolated *Salmonella* were sensitive to Sulphamethoxazole and (100%) to Gentamycin, Colistin Sulphate, and Neomycin, followed by intermediate susceptibility to Enrofloxacin. Moreover, E.coli isolates were more resistant to Enrofloxacin (80%) and Sulphamethoxazole (60%) than *Salmonella*. High rate of antimicrobial resistance of *E.coli* isolates was to amoxicillin (100%), Ampicillin (100%), and Tetracycline (100%) followed by Oxytetracyclin (80%) (**Table 1**).

Table 1:-Antibiogram profile of *salmonella* isolates from broiler chickens

Isolates	Resistance	Intermediate	Sensitive	
1	Amp, E, Aml	Enr, Te, Ot	Ct, N, Sf, Do, G, Sh, S	
2	Amp, Aml, E	Te, Enr, Ot	Ct, G, Sh, S, N, Sf, Do	
3	Amp, Aml, Sh, Ot, S, Te, E, Enr, Sf, Do	Enr	G, N, Ct	
4	Amp, Aml, Sh, Ot, S, Te, E, Do		G, N, Sf, Ct	

Keys:-

Amp=Ampicillin (10 µg), Aml=Amoxicillin (10 µg), Te= Tetracycline (30µg), E=Erythromycin (15 µg), Sh=
Spectinomycin (100µg), Ot= Oxytetracyclin (30µg), S= Streptomycin (10µg), G= Gentamycin
(10µg), Ct= Colistin Sulphate (25µg), Do= Doxycycline (30µg), Sf= Sulphamethoxazole (25µg),
Enr = Enrofloxacin (5µg) N= Neomycin (30µg)

A good sensitivity pattern of E.coli isolated has been reported to Colistin sulphate (100%) followed by Gentamycin (40%) and Sulphamethoxazole (40%). Furthermore, multi-drug resistance to < 2 antibiotics was also recorded. Where the result indicated that the frequency of both multi-drug resistance of Salmonella and E.coli was (75%) and (100%), respectively (**Table 2**).

Table 2:-Antibiogram profile of <u>*E.coli*</u> isolates from broiler chickens

Isolates	Resistance	Intermediate	Sensitive
1	Amp, Ot, S, Te, G, Sh, Aml, Enr, Sf, Do, E	N	Ct
2	Amp, Ot, S, Te, Sh, Aml, N, Enr, Sf, Do, E	G	G, Ct
3	Do, E, Ot, S, Te, G, Enr, Amp, Enr, Sh, Aml, N	Enr, Ot	Sf, Ct

	4	Amp, Ot, S, Te, E, Aml, N, Enr, Do		Sh, Sf, Ct
	5	Amp, Te, Aml, E	S, (G, Sh, N, Do, Ct, Sf
17				

Keys; Amp=Ampicillin (10 µg), Aml=Amoxicillin (10 µg), Te= Tetracycline (30µg),E=Erythromycin (15 µg), Sh= Spectinomycin (100µg), Ot= Oxytetracyclin (30µg),Streptomycin (10µg), G= Gentamycin (10µg), Ct= Colistin Sulphate (25µg),Do= Doxycycline (30 µg),Sf= Sulphamethoxazole (25 µg), Enr = Enrofloxacin (5µg),N= Neomycin (30µg).

Discussion:-

One hundred percent resistance of both isolates to B- lactam group (Amoxicillin and Ampicillin) was found and that was in agreement with (Guerra *et al*, 2003; Abd –El tawab *et al*, 2015). Similarly, 100% resistance to Ampicillin was recorded in Eygpt, 3% in Brazil, and 10% in Chile (Amàbile- Cuevas *et al*, 2010). Ampicillin resistance in *Salmonella* and *E.coli* isolates coincided with the results of (Soomro et al, 2009; Suresh et al, 2006). On the other hand, this findings were not in agreement with a study conducted by (Eissa, 2016), in camel, where isolated Salmonella showed high sensitivity to Ampicillin (100%) and Amoxicillin (100%). Interestingly, all isolates which were resistant to Ampicillin exhibited also resistance to Amoxicillin. This may be as a result of that both agents are pharmacological rela (Molla et al, 2003).

The resistance of some Salmonella and E.coli isolates to Streptomycin was (50%) and (80%), respectively. The resistance to tetracycline was also reported to be 50% in Salmonella and 100% in Ecoli. The resistance to these antimicrobial was previously demonstrated by (Castanon, 2007; Kilonzo-Nthenge et al, 2013; Phagoo and Neetoo, 2015). Resistance to tetracycline has been reported to be genetically associated with mobile plasmids or transposons (Phagoo and Neetoo, 2015). In addition, a study carried out by (Chopra and Roberts, 2001) indicated that the resistance to Tetracycline can result in inhibiting the synthesis of bacterial protein through preventing t-RNA attachment to ribosome. Furthermore, Tetracycline has been used in production animals such as poultry from one day to old broilers. Thus, the resistance developed as a result of the frequent exposure to this antibiotic during the growth period (Soomro et al, 2009). This can also be due to sub active dose and extensive use of antibiotics in poultry farms (Phagoo and Neetoo, 2015).

Macrolide antibiotics (Erythromycin) have been known for a long time for their use as therapeutic agents and growth promotion. Therefore, the resistance of some isolates to Erythromycin has been expected by investigators and may be attributed to the long term use as a growth promoter (Wanger and Cerniglia, 2005).

This study also revealed resistant of E.coli to Floroquinolones (Enrofloxacin). It should be noted that the resistant of Floroquinolones had been approved in 1990. Data from 1991 to 2000 showed differences in the resistance rate of Enrofloxacin from country between countries over time (Guardabassi et al, 2008). In Spain, the resistance of Enrofloxacin increased from 10.3% in 1991 to 41.9% in 2000. In 2002, the Enrofloxacin resistance ranged from 5% to 28.7% in Spain, Portugal, and Thailand (Guardabassi et al, 2008). On the other hand, a study was conducted by (Ngeleka et al, 2002) recommended the Enrofloxacin as the first drug of the treatment of Colibacillosis. A resistance rate of 27.9% for Enrofloxacin was reported in *Salmonella Enteritidis* in 2011, and 43% in *E.coli* in 2012 (Nunes Medeiros et al, 2011; Gregova et al, 2012). In our study, the percentage of Enrofloxacin resistance dramatically increased to 80% in the isolates of *E.coli*.

Although all of isolated *Salmonella* were highly sensitive to Gentamycin (100%) and Colistin Sulphate (100%) which may be attributed to the uncommon use of theses antibiotics in veterinary field (**Miranda** *et al*, **2010**; **Habrun** *et al*, **2010**), a slight decrease for the sensitivity of Gentamycin was observed with 60% of *E.coli* isolates

The results also showed high multi- drug resistance of *Salmonella* and *E.coli* isolates to more than two antimicrobials. This probably due to the misusage of antibiotics administrated during the production stage (**Miranda** *et al*, **2010**). In addition, in developing countries, it is very easy for both patients and farmers to obtain antibiotics from the pharmacist without a medical supervision. The emergence of multi-drug resistance organisms has been considered to be a critical issue especially in the case of the management of diseases caused by these organisms (**Tajabakhsh** *at al*, **2015**). Since it is difficult to treat infection with multi-drug resistant pathogens in compare to the susceptible one (**Akbar-Anal**, **2013**). This resistance was mostly to for antimicrobial used extensively as feed additives or therapeutics (**Tajabakhsh** *et al*, **2015**)

The frequency of multi-drug resistant of *Salmonella* was 100%. The same result was recorded in another country, which is Nepal (**Shrestha** *et al*, **2010**). The presence of multi-drug resistant *Salmonella* can be a serious issue because it has been considered one of the most important Zoonotic pathogens and that can affect the treatment of human Salmonellosis (**Molla** *et al*, **2003**; **Kilonzo-Nthenge** *et al*, **2008**). Significantly, multi drug resistant *Salmonella* has raised the food safety concern (**Akbar and Anal**, **2013**). In terms of *E.coli*, the presence of multi-drug resistant *E.Coli* has been taken as a good biomarker for determining the emergence of antimicrobial resistance (**Von Baum and Marre, 2005**; **Miranda et al**, **2010**). *E.coli* is known by its ability to transfer antimicrobial resistant between individual bacteria (**Von Baum and Marre, 2005**). Additionally, multidrug resistance of *E.coli* isolates could have resulted from the co-selection of resistance determinants, since the exposure of bacteria to one type of antibiotics may lead to resistance to other type of antibiotics without prior exposure (**Miranda et al, 2010**).

Conclusion:-

The study presented clear evidence on the emergence of drug resistant bacterial pathogens in veterinary field. It was found that many antibiotics have become not efficient against *Salmonella* and *E.coli* infections. Therefore, the sub-therapeutic and non- therapeutic use of antibiotics in veterinary medicine should be controlled. In our study, all of *salmonella* and *E.coli* isolates were sensitive to Colistin, which might make it the drug of choice for treatment of *salmonella* and *E.coli* infection. This study also developed the hypothesis that theses drug –resistant pathogens can transfer to the carcasses and subsequently to human via contaminated carcass processing, or meat consumption. This can lead us to another critical point at which the cost of health serving due to antibiotics resistance is expected. Food safety research should be conducted to monitor the presence of theses pathogens and reduce their impact on public health.

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