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Research paper

Efficacy of carvacrol oil against common broilers chickens enteric pathogens.

Ismail Radwan [1], Ahmed Orabi [2], Saber El-Hanbally [3]*, Hanaa A. Mabrouk [4]

[1] Department of Microbiology, Faculty of Veterinary Medicine, Beni-suief University, Egypt
[2] Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt
[3] Department of Pharmacology, Faculty of Veterinary Medicine, Sadat City University, Egypt
[4] Animal Health Research Institute, Al-Fayoum Lab, Egypt
**Corresponding author E-mail: elhanbally@yahoo.com*

Abstract

Gastrointestinal tract acts as a selective barrier for the broilers, however a wide range of factors associated with diet and infectious disease agents can negatively affect the delicate balance among the components of the chicken gut and, as a result, affect health status and production performance of birds in commercial poultry industries. Our investigation aimed to determine the incidence of the most common broilers chicken enteric pathogens as Salmonella, *E.coli and C.perfringens* and also measure efficacy of carvacrol oil on these pathogens. 250 internal organs were analyzed for enteric pathogens in (table 1, 2) which revealed high incidence of *E.coli* (n=18) followed with Salmonella (n=10) and *C.perfringens* (n=8).Serotyping of Salmonella isolates showed that the predominant serovars is *S.Kentuky* (n=3) then *S.Typhimurium* and *S.Infants* (n=2), while O114:K90 O78:K80 O25:K11 were the predominant *E.coli* serotypes (n=4) for each one. Antibiogram revealed that isolates were resistance to Cefotaxime, Amoxicillin, Doxycycline and Enrofloxacin. Results indicated that, MIC of most isolates lowered after 24hr exposure to 0.001% of carvacrol and the growth of tested isolates was inhibited at 0.1% carvacrol concentration.

Keywords: *Carvacrol Oil; Enteric Pathogens*

1. Introduction

The enteric health of growing poultry is imperative to success of the production. Broilers are very efficient at both growth and feed conversion rate. Any Enteric disorders affect poultry lead to high economic losses due to increased mortality rates, decreased weight gain, increased medication costs, and increased feed conversion rates. Several pathogens are incriminated as possible causes of enteric disorders as *E.coli*, Salmonella and Clostridium either alone or in synergy with each other. Avian collibacillosis causes significance economic losses either primary or secondary infection to broilers chickens as adhesion and proliferation of avian pathogenic *E.coli* "APEC" is mainly due to presence of virulence factors of which antimicrobial resistance is one of the most important (Carli et al., 2015). Domestic poultry constitutes the single largest reservoir of Salmonella serovars. During the last years, the number of Salmonella infection in poultry flocks and human being has been increased substantially in several European Countries, USA and Egypt (Desmidt et al., 1998).Necrotic enteritis (NE) is an economically important enteric disease in poultry. Clostridium perfringens is a gram-positive anaerobic bacterium that is able to form spores. It is widespread in the environment (e.g. in soil and sewage) and is commonly found in the intestines of animals, including humans, where it is pathogenic in certain circumstances, outbreaks of necrotic enteritis, caused by *C.perfringens*, have been frequently reported in chickens throughout the world (Ahsani et al., 2011).The spread of antibacterial resistance has an additional implication since; it may assist in the establishment and persistence of pathogenic microorganisms in the host (Nolan et al., 1991).Increased antimicrobial bacterial resistance guided scientific attention to plant extract and essential oils "Eos" as carvacrol (Ben Arafa et al., 2006), which might be substitute cure to synthetic chemical compounds (Al Laham and Alfadel., 2013). Carvacrol (2-methyl-5-isopropylphenol) is a monoterpene phenolic constituent of essential oils of various aromatic plants (Amiri., 2012).This work was designed to study susceptibility of E.coli, Salmonella, *C.Perfringens* recovered from broilers chickens enteric disorders to some antimicrobial agents using MIC technique with and without carvacrol as one of the most important essential oils to determine carvacrol efficacy against tested enteric pathogens as antibiotic alternative agent to control emergence of antibiotic resistance .

2. Material and methods

2.1. Samples

Internal organs (n=250) were collected from different broilers farms at different ages suffered from enteric disorders and cultured within 24 hr. from collection.

2.2. Identification of enteric pathogens

Under complete sterile condition each cultured directly on MacConkey's agar for detection of *E.coli* while detection of Salmonella was carried out according to ISO 6579: (2002). The suspected colonies were picked up and examined microscopically by Gram's stain before being transferred into semisolid and slope agar for

preservation and further identification biochemically (Quinn et al., 2011) and serologically (Kauffmann and Das Kauffmann., 2001),while samples were cultured on (TSC Agar, Oxoid) plates and incubated in an anaerobic chamber at 37 ¡C for 24 h. Black colonies, presumed to be *C.perfringens*, were identified by biochemical tests and semi-antitoxin petri method using specific alpha toxin antisera and egg yolk agar (Russo and Gorbach ., 1987).

2.3. Antiprogram and (MIC) minimum inhibitory CONC

Antibiogram disk diffusion technique was adapted according to (CLSI., 2017),while MIC was applied according to (Koneman et al., 1997), as all examined strains were tested against selected antibiotics obtained from Sigma –Aldrich pharmaceutical grade before and after growth for 24 hr. on Muller Hinton agar containing the concentration of carvacrol at which no growth inhibition was detected .

3. Results and discussion

The resistance originating in poultry strains can be transmitted to human and this is a potentially a serious public health hazard and not only because of the consequent problems regarding therapy, but also because of the risk of resistance spreading to other enteric organisms including normal flora through the vivo passage of plasmids (Tassios et al., 1997). The antibiogram of a pathogen could be variable from place to another and from case to another. This could be attributed to the wide use of antibacterial agents in poultry industry in Egypt which may produce new resistant bacteria. One of the steps in controlling salmonellosis is the use of the appropriate antibacterial agent. The kind of this agent should better be selected on the basis of its sensitivity which could be detected by disk diffusion method (Finegold and Martin, 1982). In the current investigation, 250 internal organs were analyzed for enteric pathogens in (table 1, 2) which revealed high incidence of E.coli (n=18) followed with Salmonella (n=10) and *C.pergringens* (n=8).Serotyping of Salmonella isolates showed that the predominant serovars is *S.Kentuky* (n=3) then *S.Typhimurium* and *S.Infants* (n=2), while O114:K90 O78:K80 O25:K11 were the predominant *E.coli* serotypes (n=4) for each one. Antibiogram in table (3) showed that examined isolates were resistance to Cefotaxime, Amoxicillin, Doxycycline and Enrofloxacin. The extreme resistance of isolates to ampicillin and amoxicillin is attributed to the wide use of these two antibiotics for treatment and prophylaxis of poultry diseases during the last two decades. The alarming ability of Salmonella to require persistent high level resistance to the clinically most revalent antibacterial has become increasingly important and serious problems (Pang et al., 1995).

Collibacillosis is known to contribute significantly to increased mortality and economic losses in the poultry industry45. As a result, antimicrobials, sometimes at sub-therapeutic concentrations, are often included in feed given to food animals to prevent disease, reduce mortality and morbidity, enhance feed conversion efficiency and improve growth rates (Aarestrup et al., 2001).

Table 1: Incidence of Broilers Enteric Pathogens

Organ	n=samples	n=Salmonella	n=*E.coli*	n=*C.pergringens*
Liver	50	3(6%)	5(10%)	0
Yolk Sac	40	2(5%)	7(17.5)	0
Lung	40	1(2.5)	2(5%)	0
Intestine	100	4(4%)	2(2%)	8(8%)
Spleen	20	0	2(10%)	0
Total	250	10	18	8

Table 2: Serotyping of Broilers Enteric Pathogens

Organ	n= Salmonella	Serotype	n= *E.coli*	Serotype
Liver	3	*S.Kentuky*(n=2) *S.Infants*(n=1)	5	O44:K74(n=2) O114:K90(n=2) O119:K69(n=1)
Yolk Sac	2	*S.Enteritides*(n=1) *S.Heidelberg*(n=1)	7	O78:K80(n=3) O25:K11(n=2) O114:K90(n=2)
Lung	1	*S.Infants*(n=1)	2	O25:K11(n=2)
Intestine	4	*S.Typhimurium*(n=2) *S.Kentuky*(n=1) *S.Agona*(n=1)	2	O126: K71(n=1) O78: K80(n=1)
Spleen	0	-	2	O126: K71(n=2)

Table 3: Resistance Pattern of broilers enteric pathogens to the used antibiotics.

Antimicrobial agents	Salmonella Resistance patterns (n=10)	*E.coli* Resistance patterns (n=18)	*C.perfringens* Resistance patterns (n=8)
Sulphamethaxole + Trimethoprim	6/10	10/18	3/8
Lincospectin	2/10	5/18	5/8
Doxycyclines	8/10	6/18	6/8
Ampicillin	8/10	10/18	5/8
Penicillin	5/10	9/18	2/8
Amoxycillin	6/10	8/18	5/8
Chloramphenicol	2/10	4/18	8/8
Gentamicin	1/10	5/18	3/8
Enrofloxacin	6/10	6/10	8/8
Cefotaxime 30 µg	5/10	9/18	8/8

A high number of *C.perfringens* in the intestinal tract and associated necrotic lesions have been detected in poultry flocks worldwide that suffer from necrotic enteritis (Tsai and Tung, 1981). A better understanding of conditions that favor the proliferation of *C.perfringens* is important in determining the cause and spread of necrotic enteritis (Paulus and Ruckebusch, 1996). Factors that predispose flocks to necrotic enteritis includes management stress, changes in dietary formulation, alteration of feeding programs, and subclinical intestinal coccidiosis (Shane et al., 1985).*C.perfringens* is commonly found in the intestinal tract of poultry, but the occurrence of the poultry disease, necrotic enteritis, is sporadic (Cowen et al., 1987). A complete understanding of the factors contributing to infection of the intestinal tract and subsequent events leading to necrosis is lacking. Examination of the intestinal tract of affected birds in flocks with this disease and in controlled studies shows that high numbers of *C.perfringens* cells are present and are intimately associated with damaged tissue. Because these high numbers are not generally found in the intestinal tract of birds from healthy flocks, conditions favoring proliferation of *C.perfringens* in the intestinal tract appear to be essential for disease outbreaks (Tschirdewahn et al., 1991).

Carvacrol is an essential oil fraction of oreganum and thyme having antimicrobial activities against different pathogens (Si et al., 2006). In the present study, different concentration of carvacrol used to determine its inhibitory effect of common enteric pathogens of broilers and determination of MIC of selected antibiotics before and after exposure of recovered pathogens to carvacrol oil as described in (table 5-8) which revealed that 0.1% carvacrol conc. has potent antibacterial effect against Salmonella, *E.coli* and *C.pergringens* and also using carvacrol potentiate effect of antibiotics and decrease its MIC. Essential oils and other plant extracts evoked interest owing to their potential uses as alternative remedies for the treatment of many infectious diseases, some of these essential oils show inhibitory activity against multidrug resistant bacteria as essential oils and its anti-quorum sensing activity that might be important in reducing virulence and pathogenicity of drug resistant bacteria (Oliveira and Cunha, 2008).In the present study decreasing MIC of the present four used antibacterial drugs after exposure to 0.001% of carvacrol might be attributed to repression of autoinducers "AIs" which are extracellular signaling system of stimuli and reflexes orchestrates important events related to bacterial virulence (Zhu et al., 2016).

4. Conclusion

Carvacrol oil consider as pathogens inhibitory agent in broilers chicken suffered from enteric disorder with failure antibiotic control as it has direct antimicrobial effect and also can potentiate effect of antibiotic by decreasing its minimum inhibitory concentration .

Table 5: MIC for Salmonella Serovars before and after treatment with Carvacrol

MIC for Salmonella serovars								
n=10	Before carvacrol treatment				After carvacrol treatment			
	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin
1.S.Kentuky	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
2.S.Kentuky	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
3.S.Kentuky	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
4.S.Enteritides	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
5.S.Heidelberg	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
6.S.Typhimurium	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03
7.S.Typhimurium	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
8.S.Infants	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
9.S.Infants	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
10.S.Agona	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03

Table 6: MIC for *E.Coli* Serotypes before and after treatment with Carvacrol

MIC for *E.coli* serotypes								
n=18	Before carvacrol treatment				After carvacrol treatment			
	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin
1. O44:K74	0.24	0.25	0.23	0.25	0.03	0.04	0.03	0.03
2. O44:K74	0.26	0.25	0.23	0.25	0.04	0.03	0.04	0.03
3. O114:K90	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
4. O114:K90	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
5. O114:K90	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
6. O114:K90	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03
7. O119:K69	0.25	0.25	0.25	0.27	0.03	0.03	0.04	0.03
8. O78:K80	0.25	0.25	0.25	0.25	0.05	0.03	0.03	0.03
9. O78:K80	0.25	0.25	0.25	0.27	0.03	0.03	0.03	0.03
10. O78:K80	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03
11. O78:K80	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
12. O25:K11	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
13. O25:K11	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
14. O25:K11	0.1	0.2	0.1	0.2	0.02	0.2	0.11	0.02
15. O25:K11	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03
16. O126	0.25	0.25	0.25	0.25	0.03	0.03	0.03	0.03
17. O126	0.3	0.3	0.3	0.3	0.12	0.12	0.12	0.12
18. O126	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2

Table 7: MIC for *C.Perfringens* before and after treatment with Carvacrol

MIC for *C.perfringens*								
n=8	Before carvacrol treatment				After carvacrol treatment			
	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin	Doxycycline	Cefotaxime	Amoxicillin	Enrofloxacin
1. C.perfringens	0.4	0. 7	0.3	0.8	0.04	0.07	0.02	0.08
2. C.perfringens	0.5	0. 7	0.3	0.9	0.05	0.07	0.03	0.08
3. C.perfringens	0.4	0. 6	0.3	0.8	0.3	0.5	0.12	0.06
4. C.perfringens	0.4	0. 7	0.3	0.8	0.04	0.07	0.02	0.08
5. C.perfringens	0.5	0. 7	0.3	0.9	0.05	0.07	0.03	0.08
6. C.perfringens	0.4	0. 8	0.3	0.9	0.03	0.07	0.03	0.09
7. C.perfringens	0.4	0. 6	0.3	0.8	0.03	0.06	0.03	0.07
8. C.perfringens	0.6	0. 7	0.3	0.8	0.05	0.06	0.03	0.07

Table 8: Growth Inhibition Concentration of Carvacrol against broilers enteric pathogens

Pathogens	Concentration of carvacrol		
	0.1 %	0.01%	0.001%
Salmonella serovars	-	+	+
E.coli serotypes	-	-	+
C.perfringens	-	+	+

+ = growth - = no growth

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