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Phenotypic and Genotypic correlation between avian pathogenic and fecal Escherichia coli isolated from broiler chickens

A Thesis
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For the degree of philosophy doctorate (PhD)
Veterinary medical science
(Bacteriology, Mycology and Immunology)

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(2022)

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List of Abbreviations

AFEC	Avian fecal E. coli
AM	Amikacin
AMC	Amoxicillin-clavulanic acid
AML	Amoxicillin
ampC	ampicillinase C plasmid-mediated inducible ß-lactamase
APEC	Avian pathogenic Escherichia coli
AST	Antimicrobial susceptibility testing
ATM	Aztreonam
Bp	Base pair
CAZ	Ceftazidime
CFU	Colony forming unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standard Institute
CR	Congo red
CRO	Ceftriaxone
CT	Colistin sulphate
CTX	Cefotaxime
DNA	Deoxyribonucleic Acid
DO	Doxycycline
DW	Distilled water
eaeA	Attaching and effacing intimin gene A
EO	Essential oils
EPEC	Extraintestinal pathogenic E. coli
EPEC	Enteropathogenic E. coli
ExPEC	Extra-intestinal pathogenic E. coli
ESBLs	Extended spectrum beta lactamases
FIC	fractional inhibitory concentration index
fimH	Fimbrin D-mannose specific adhesin
FO	Fosfomycin
G	Gram
GIT	gastrointestinal tract

HBSS	*Hanks balanced Salts Solution*
IFNG	*Interferon gamma*
IL	*Interleukin*
int1	*Integrase genes,*
iss	*Increased serum survival gene*
IU	*International Unit*
K	*Kanamycin*
LB	*Luria Bertani agar:*
MBC	*Minimal bacteriostatic concentration*
MBIC	*Minimal biofilm inhibitory concentration*
Mcr1	*plasmid-mediated resistance to colistin*
MDR	*Multi drug resistant*
Mg	*Milligram*
MIC	*Minimal inhibitory concentration*
Ml	*Milliliter*
Mm	*Millimeter*
m-PCR	*Multiplex Polymerase Chain Reaction*
MR test	*Methyl red test*
MSHA	*mannose-sensitive haemagglutinin*
MY	*Lincomycin*
OEO	*oregano essential oil*
OMVs	*outer membrane vesicles*
pic	*Serine protease pic autotransporter Involved in intestinal colonization,*
qRT- PCR	*Quantitative real time polymerase chain reaction*
S	*Streptomycin*
Spp	*Species*
SXT	*Sulfamethoxazole/ Trimethoprim*
Tsh	*Temperature sensitive heamagglutinin*
TSI	*Triple sugar iron agar media*
V. P. test	*Voges Proskauer test*
YESCA agar	*Yeast extract casamino acids agar*

6. Summary:

In the current study bacteriological examination of samples collected from broiler chicken's intestine and internal organs revealed that the overall isolation rate of *E. coli* from the intestine of apparently healthy birds were 53% (80/150). While the prevalence of isolation of avian pathogenic *E. coli* (APEC) from different lesions of diseased broiler were 60% (90/150). Chickens. Regards to the site of systemic isolation results showed that most of *E. coli* isolates were obtained from liver (32.7%) followed by heart blood (24.7%) and yolk sac (2.6%).

All the recovered *E. coli* isolates were subjected to the phenotypic in-vitro pathogenicity based on Congo Red binding assay, assessment of biofilm formation ability, Hemolysis assay and serum resistance assay.

CR-binding assay indicated that 52 / 80 (65%) (AFEC) isolates and 50/90 (55%) (APEC) isolates were positive.

Biofilm formation ability was detected in 35 out of 80 (43.7%) of the intestinal isolates and 30 out of 90 the systemic isolates (33.3 %) were able to generate a moderate or strong biofilm.

In this study, 75 % of the avian intestinal *E. coli* isolates and 90% of the avian pathogenic *E. coli* showed α-haemolysis on sheep blood agar 5%.

Serum resistance was seen in 32 (40%) of avian intestinal *E. coli* isolates and 59 (65.5%) of the systemic isolates.

Serogrouping of 28 *E. coli* isolates which were selected positive for all the in-vitro phenotypic virulence markers (14 AFEC and 14 APEC)

revealed that the most prevalent fecal *E. coli* serogroups was O1 followed by O128 and O158 with the prevalence of 28.5 %, 21.5% and 14.5% respectively, While the most prevalent systemic *E. coli* serogroups isolated was O119 and O25 with the prevalence of 21.5% for each followed by O86 (14.5%) and O1 (7%)

Antibiogram of *E. coli* isolates were evaluated and the result confirmed that all the tested biofilm forming isolates (100%) either intestinal *E. coli* (n=25) or systemic *E. coli* (n=25) were resistant to three or more classes of antibiotics and were considered MDR. High resistance was recorded to amoxicillin (100% for each) and Ceftriaxone (100% for each) followed by Lincomycin (96% and 100%), Kanamycin (96% and 92%), streptomycin (96% for each) and Sulfamethoxazole/ Trimethoprim (96% and 100%), ciprofloxacin (88% and 96%), Doxycycline (88% and 76%), Colistin sulphate (80% and 68%), aztreonam (72% and 80%), Ceftazidime (68% and 80%), Amoxicillin-clavulanic acid (68% and 80%) and Cefotaxime (64% and 54%). While sensitivity was recorded toward Amikacin (80% and 76%) and Fosfomycin (52% and 60%) of the (AFEC) and (APEC) respectively.

screening of some resistance and virulence genes was investigated in 12 MDR of our isolates (six AFEC and six APEC).

Resistance genes *Int*1 and *amp*C were detected with the prevalence of 100% in both (AFEC) and (APEC) isolates while *Mcr*1 gene was found in 100% of APEC and (83.3%) of AFEC strains.

The intended virulence genes (*fim*H, iss and *eae*A) were detected in all the studied isolates (100%), while (pic and tsh) genes were detected with prevalence of (16.7%) in both AFEC and APEC isolates.

In this study the antibacterial effect of two essential oils (cinnamon and carvacrol) were investigated for their antimicrobial activity against 50 MDR *E. coli* isolates and the result showed that they were able to completely inhibited the growth of all the tested isolates at concentration of 1% and 0.5% respectively.

Results of experimental infection proved that fecal *E. coli* isolates were able to produce different signs of colibacillosis in broiler chicken model under the experiment, with high re-isolation rate from different internal organs. using of amino acid mixture rich in glycine can minimize the negative effects of the experimental *E. coli* infection.

The immune status of the chickens under experiment evaluated by different parameters. the recorded results for some selected avian cytokines using qRT- PCR, revealed that there was fold change increasement in treated groups with amino acids rich in glycine T1: T2: T3, while There is confinement of these mediators in T4, T5 and T6 which reflect on immune response against infectious agents

Regarding phagocytic count and its mediators, there were augmentation in the cells count from in T1, T2 and T3 respectively in comparison with T4, T5 and T6 which may indicate the activation of macrophages cells by action of dietary supplementation of amino acid mixture rich in glycine.