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Molecular characterization of bacteriophage and its effect on antimicrobial resistant bacteria

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LIST OF ABBREVIATIONS

AEEC	Attaching and effacing *Escherichia coli*
APCs	Antigen presenting cell
APEC	Avian pathogenic *Escherichia coli*
BPW	Buffered peptone water
cDNA	Complementary deoxy ribonucleic acid
CLSI	Clinical and laboratory standards institute
CFU	Colony forming unit
DNA	Deoxy ribonucleic acid
***eae*A**	Gene encodes intimin which responsible for attaching and effacing
EHEC	Enterohemorrhagic *Escherichia coli*
ESBLs	Extended spectrum beta lactemas
IBV	Infectious Bronchitis virus
ICTV	International Committee on the Taxonomy of Viruses
***inv*E/A**	*Salmonella* invasive gene
MDR	Multidrug resistant
***mec*A gene**	Gene carry resistance to methicillin, penicillin
MRSA	Methicillin -resistant *Staphylococcus aureus*
NTS	Non-typhoidal *Salmonella*
PCR	Polymerase chain reaction
PFU	Plaque forming unit

RFLP	Restriction fragment length polymorphism
SPIs	*Salmonella* pathogenicity island gene
STEC	Shiga toxin producing *Escherichia coli*
***Stx*1**	Shiga toxin group one gene harpered in Shiga toxin producing *Escherichia coli*
***Stx*2**	Shiga toxin group two gene harpered in Shiga toxin producing *Escherichia coli*
TEM	Transmission electron microscopy
TSB	Tryptone soya broth

7-SUMMARY

In this study 80 diseased chickens of varying ages (one-day old to 45 days old) were collected from 23 broiler farms in Luxor Governorate, in the South of Egypt.

17 *E. coli* were isolated with percentage 73.9% (17/23), the most predominant serotypes were O_{142}- 11.7% (2 strains out of 17), O_{27}- 11.7%(2 strains out of 17), O_{114} - 5.8% (1 strains out of 17), O_{26} -5.8% (1 strains out of 17), O_{125} -23.5(4 strains out of 17), O_{126} -17.6% (3 strains out of 17), O_6 - 5.8% (1 strains out of 17) , O_{78}-5.8% (1 strains out of 17) ,*E. coli* O_{146}-5.8% (1 strains out of 17) and *E. coli* O_{86}a -5.8% (1 strains out of 17). *E. coli* isolates showed high resistance to Amoxicillin 10µg (100%), tetracycline 30µg (94.4%), Streptomycin 10µg and Enrofloxacin 5µg (83.3%), Neomycin 30µg (77.7%), Chloramphenicol 30µg (61.1%), each of Florfenicol 30µg and Ofloxacin 5µg (55.5%), Norfloxacin 10µg (50%). On the other hand, lower rates of resistance were observed for Gentamycin (22.2%), and Cefotaxime (16.7%), while all isolates were sensitive to Nitrofurantoin 300µg (100%).

The incidence rate of (*eae* A) gene were 47% ,11.7% for *Stx*1gene and 23.5% for *Stx*2 gene .

The incidence of *Salmonella* in broiler chicken, were 9/23(39.1%) and the the most predominant serotypes were *Sal.* Typhimurium (n=1) *and Sal.* Enteritidis (n=2)**,** *Sal.* Kentucky (n=2)**,** *Sal.* Blegdam (n=1)**,** *Sal.* Montevideo (n=1), and *Sal.* Gueuletape (n=2). *Salmonella* isolates, higher resistance was exhibited to Amoxycillin 10µg (87.5%), Neomycin 30µg (75%), Streptomycin 10µg (62,5%), and Tetracycline 30µg (56.25%), while lower rates of resistance were observed for Nitrofurantoin 300µg (37.5%), each of Florfenicol 30µg, Enrofloxacin 5µg and Gentamycin 10µg (31.25%), Ofloxacin 5µg (25%), and each of Chloramphenicol 30µg, Norfloxacin 10µg, and Cefotaxime 30µg (18.75%). Several multidrug resistance (MDR) profiles to three or more antimicrobial classes were detected in 76.7% of *Salmonella* serovars.

18 bacteriophages were isolated from intestinal content with different plaque size and appearance,9 bacteriophage with clear plaque appearance were further purified.

Nine purified bacteriophages classified morphologically using Transmission Electron Microscope (TEM), and named according their appearance under TEM microscope, Phages *vB_salk1S, vB_salk 3S, vB_salNS* and *vB_EnaS* belong to the family *Siphoviridae*. While phages

*vB_Salk2M***,** *vB_SauM, vB_ EO26M and vB_ EO27M* belong to the *Myoviridae* family and *vB_ EO114* belong to *Podovireade* family.

Host range analysis using EOP indicated that the *vB_SauM* phage had plaques against one *Staph. aureus* isolate with high efficiency (EOP = 1), *vB_ EO26M* had plaques against 3 *E. coli* isolates with low efficiency (EOP = 0.012, 0.01 and 0.02) and one *Enterobacter aerogenes* isolate produced low efficiency (EOP = 0.006). The *vB_salNS* had plaques against one Salmonella isolate with high efficiency (EOP = 0.6) and low efficiency (EOP = 0.06) against another salmonella isolate. The *vB_salk3S* phage had plaques against 3 Salmonella isolates with low efficiency (EOP = 0.01, 0.025 and 0.021) while *vB_Salk2M, vB_salk1S, vB_ EO27M, and vB_EnaS* had no effect against tested isolates.

Bacteriophages were tested for survival at various temperatures, ranging from 4 °C to 80 °C. The vast majority of phages from the collection survived, to some extent, incubations at 4 °C to 60 °C, except phage vB_SauM were completely inactivated ,In most cases the phage titer dropped after 60 min incubation at the 50°C. However, four phages (vB_EO114- vB_EnaS- vB_Salk2M- vB_SalNS) showed high survivability (50–100%) at 50 °C ,also two phage (vB_SalNS- vB_Salk2M) showed high survivability (42–53%) at 60 °C, while at 70°C phage titer sharply dropped and four phages(vB*_EO114*- vB*_EO27S*- vB*_Salk1S*- vB*_SauM*) completely inactivated, more over phage vB*_SalNS* could survive even at 80 °C while vB*_EO114*- vB*_EO27S*- vB*_EnaS*- vB*_Salk1S*- vB*_Salk2M*- vB*_SauM*- vB*_Salk3S*- vB*_EO26M* completely inactivated.

The effects of high and low pH on phage virion stability were also studied. all phages were survived at PH 4:12 except phage vB*_SauM*, completely inactivated at PH 10 ,12, while none of phages survived at PH 2 except three phages

(vB*_SalNS* **-** vB*_Salk3S* **-** vB*_EO26M***).**

The virions were resistant to chloroform. However, 70% ethanol caused a titer drop of most phages, also caused complete inactivation of phages (vB*_EO114P*- vB*_EO27S*- vB*_SauM*) though the virions were sensitive for 70% ethanol.

The restriction enzyme analysis for vB*_SalNS* and vB*_Salk3S* showed that the phage DNA possess restriction site for TaqI, HindIII, the two phages have similar restriction patterns .