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Molecular characterization of bacteriophage and its effect on antimicrobial resistant bacteria

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CONTENTS

Title	pages
1-Introduction	1
2-Review of literatures	6
I-Bacteriophage	6
1. Discovery and History	6
2. Morphology and classification	7
3. Life cycle	8
4. Isolation and identification	9
5-Techniques for host range determination	10
6- Phage application to control multidrug resistant bacteria	12
II-Antibiotic resistant bacteria	20
III-<i>E. coli</i>	22
1. Prevalence in poultry	22
2. Virulence gene of <i>E. coli</i>	27
3. Economic importance	28
IV-<i>Salmonella</i>	29
1. Prevalence of <i>Salmonella</i> in poultry	29
2. Virulence gene of <i>Salmonella</i>	32
3.Economic importance	33
3-Material and methods	35

4-Results	63
5-Discussion	90
6-Conclusion	96
7-Summary	97
8-References	99
9-Arabic summary	1

LIST OF TABLES

Table no.	Title	pages
1	Type and number of examined samples	35
2	list of the source of identified bacterial strains used in the present study.	36
3	Antibiotic discs used for susceptibility test	40
4	Oligonucleotide primers for virulence genes.	42
5	Interpretations of changes of different biochemical media	48
6	Antimicrobial and zone diameter interpretation	50
7	Biochemical identification of suspected <i>Salmonella</i> isolates according to ISO.	53
8	Preparation of PCR master mix according to Emerald GT PCR	56
9	Cycling condition of different primers during PCR	57
10	Preparation of RFLP master mix	61
11	Incidence of <i>E. coli</i> and serogroups recovered from examined broilers.	63
12	Sensitivity of <i>E. coli</i> serotypes to different antibiotic agents.	64

13	The percentage of reacted <i>E. coli</i> isolates	65
14	The characterization of virulence gene among different <i>E. coli</i>	66
15	Incidence of <i>Salmonella</i> and serogroups recovered from examined broilers.	68
16	Sensitivity of <i>Salmonella</i> serotypes to different antimicrobial agents	69
17	<i>Salmonella</i> isolates reacts with antimicrobial agents	70
18	The genotypic characterization of different pathogenic islands among <i>Salmonella</i> serovars.	71
19	Bacteriophages isolated against different bacterial isolates	77
20	The physical properties and belonging family of obtained bacteriophages	78
21	The titer expressed in plaque forming unite(pfu) of different isolated bacteriophages.	81
22	The result of spot test to determine the efficacy of isolated bacteriophages against different bacterial isolates	83
23	Efficiency of plating EOP to determine the bacteriophages efficiency against the different bacterial isolates showing positive spot test.	85
24	The thermostability of isolated bacteriophages at different temperatures.	86

25	The stability of isolated bacteriophages at different PH degrees.	87
26	The effect of organic solvents on stability of isolated bacteriophages.	88

LIST OF FIGURES

Figure no.	Title	pages
1	Basic morphologies of different families of prokaryote viruses	7
2	Basic morphologies of the three families of Caudovirales	8
3	The lytic and lysogenic pathways of typical bacteriophage	9
4	Amplification of <i>eaeA</i> gene of <i>E. coli</i>	67
5	Amplification of <i>sxt1</i> gene of <i>E. coli</i>	67
6	Amplification of <i>sxt2</i> gene of <i>E. coli</i>	67
7	Amplification of pathogenicity islands gene of <i>Salmonella</i>	72
8	Different plaques shapes and size	74:76
9	TEM shapes of different phages isolated	79:80
10	Spot test determining the host range of the lytic phage against different bacteria species	84
11	Restriction digestion of phage DNA vB_ <i>Sal</i> NS (1), vB_ <i>Salk</i> 3S (2) using TaqI and HindIII	89

LIST OF ABBREVIATIONS

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

RFLP	Restriction fragment length polymorphism
SPIs	<i>Salmonella</i> pathogenicity island gene
STEC	Shiga toxin producing <i>Escherichia coli</i>
<i>Stx1</i>	Shiga toxin group one gene harpered in Shiga toxin producing <i>Escherichia coli</i>
<i>Stx2</i>	Shiga toxin group two gene harpered in Shiga toxin producing <i>Escherichia coli</i>
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 O₁₄₂- 11.7% (2 strains out of 17), O₂₇- 11.7% (2 strains out of 17), O₁₁₄ - 5.8% (1 strains out of 17), O₂₆ -5.8% (1 strains out of 17), O₁₂₅ -23.5(4 strains out of 17), O₁₂₆ -17.6% (3 strains out of 17), O₆ - 5.8% (1 strains out of 17) , O₇₈-5.8% (1 strains out of 17) ,*E. coli* O₁₄₆-5.8% (1 strains out of 17) and *E. coli* O_{86a} -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 *Stx1* gene and 23.5% for *Stx2* 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 *Podoviridae* 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 .