Shams University

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Bacteriophages targeting antibiotic resistant pathogenic bacteria in infected broiler chickens

A Thesis
Submitted for the Degree of Doctor of Philosophy in Microbiology (Virology)

By

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M.Sc. (2014)

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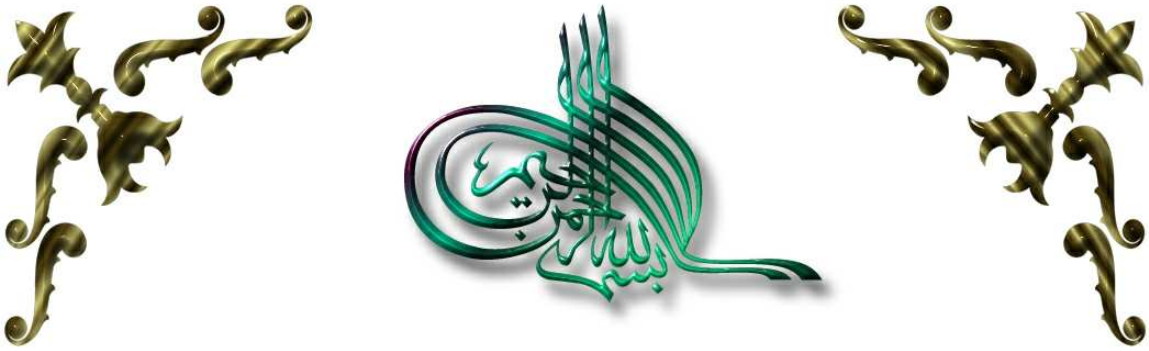

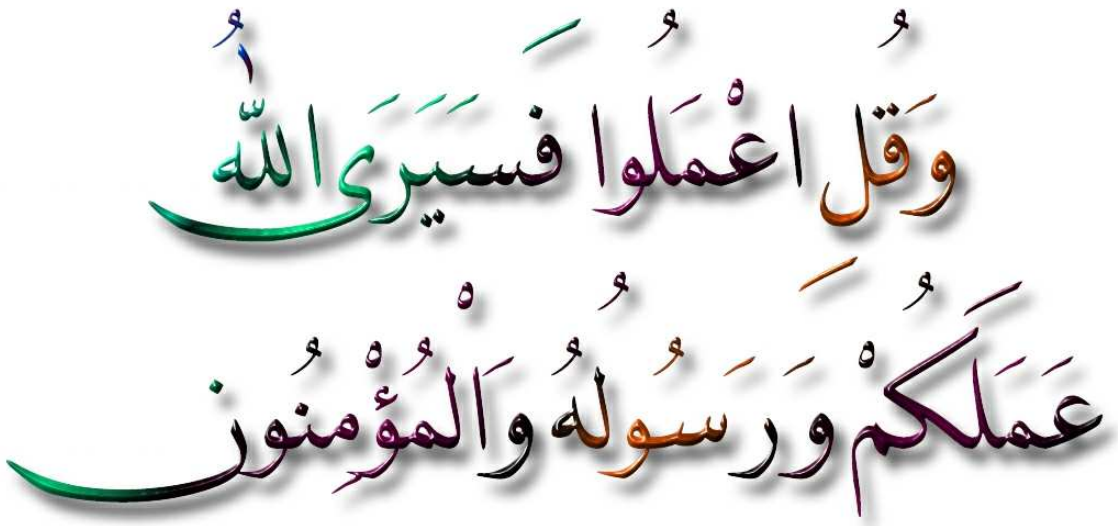

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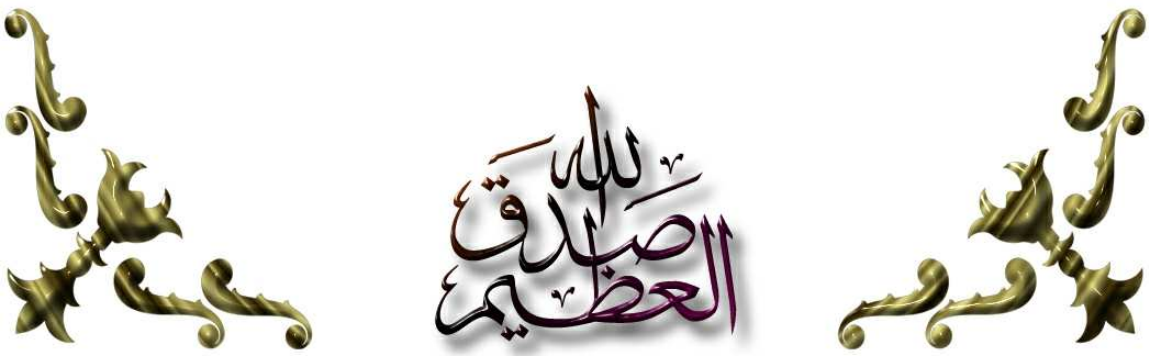

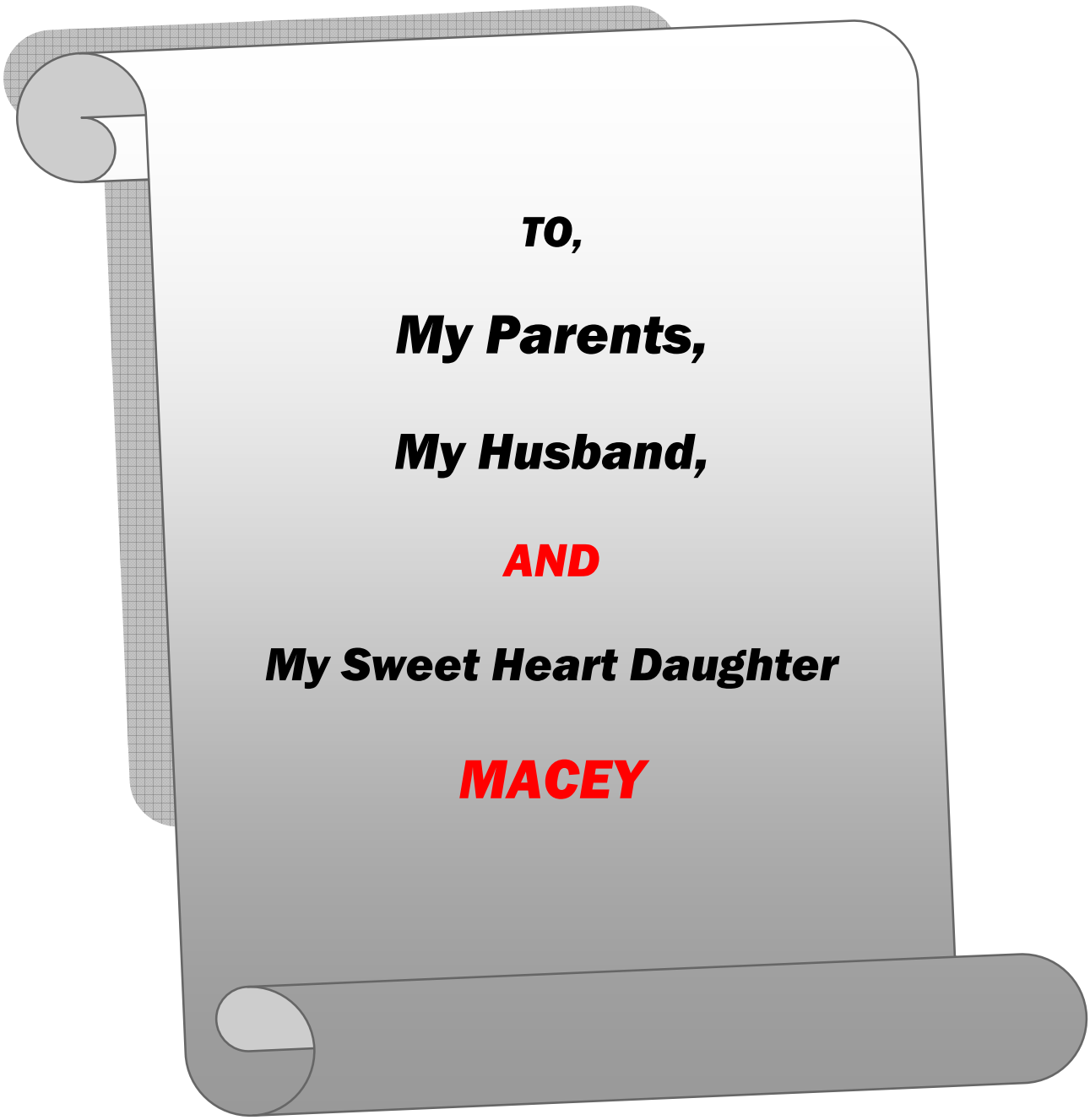
TO,
My Parents,
My Husband,
AND
My Sweet Heart Daughter
MACEY

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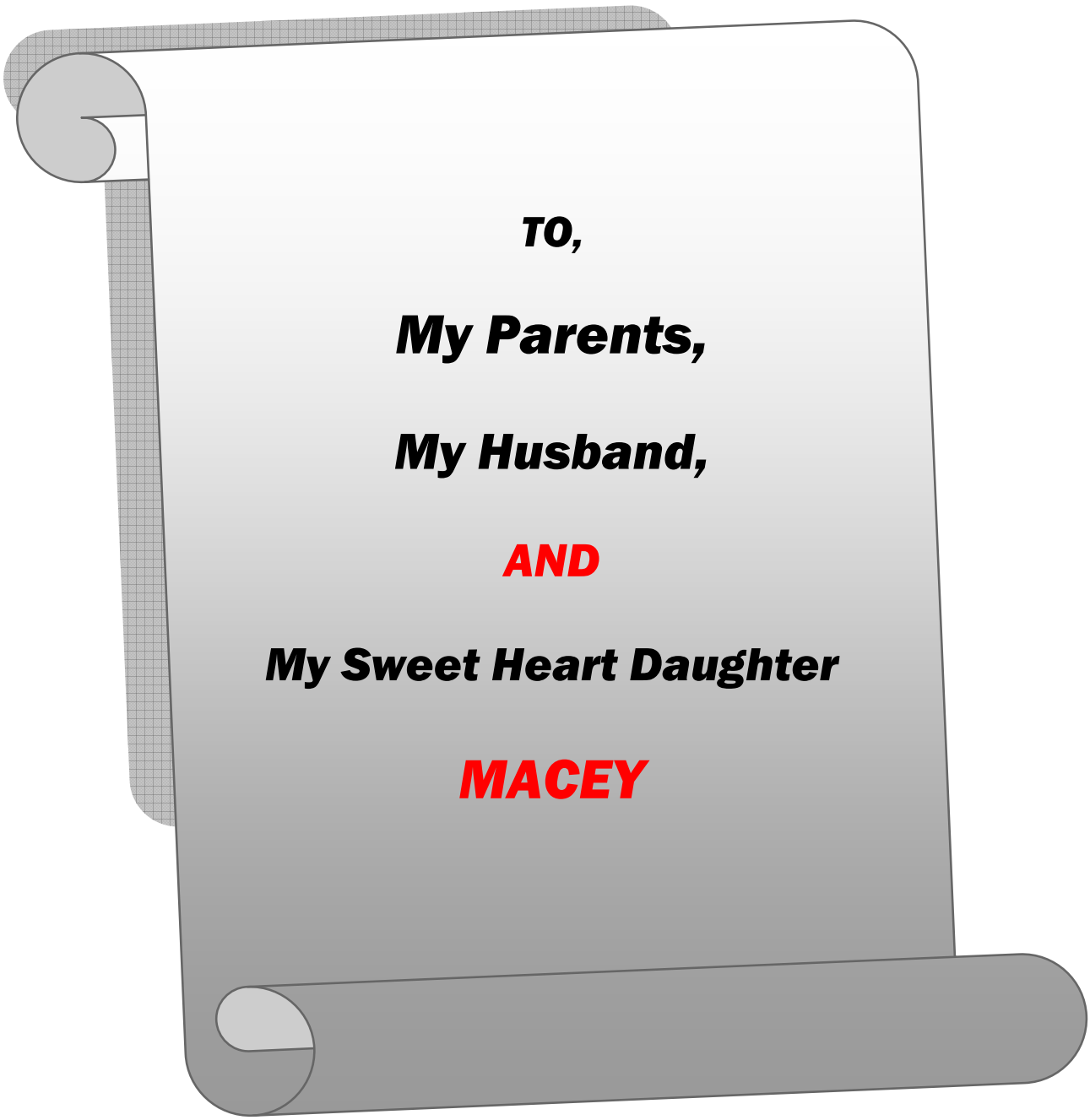
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Abstract

In recent years, the use of bacteriophages as antimicrobial agents controlling pathogenic bacteria in poultry has appeared as a promising new alternative strategy in the face of growing antibiotic resistance, which has caused problems in many fields including medicine, veterinary medicine, and agriculture. Thus, this study was conducted to investigate the prevalence and antimicrobial resistance of different pathogenic bacteria isolated from broiler chickens and the use of their bacteriophages an alternative means of controlling these pathogens. Total numbers of 86 bacterial strains were isolated from broiler chickens samples during this study. Gram-negative bacteria accounted for 87.3% (75 strains) and Gram-positive represented 12.7% (11strains). Major species were *Salmonella*, *E.coli*, *Proteus spp. Staphylococcus aureus* (4%) and *Bacillus* (1.5%). The most prevalent *Salmonella* serovars were *S. typhimurium* (7.5%), *S. enteritidis* (5.0%), and *S. kentucky* (3.0%) while the prevalence of *E. coli and Proteus sp.* was *(*14.5% and 7.5 %) respectively. Antimicrobial resistance profiles of these isolates were determined using the phenotypic agar disc diffusion method. The genes encoding resistance in the resistant strains were screened using PCR. The results showed that bacterial isolates were resistant to at least 3 tested antimicrobial. The PCR results indicated that the tested pathogenic isolates contained antimicrobial resistance gene (blaTEM), which is known to confer resistance. Moreover, the *mec*A gene was examined in methicillin resistant *S. aureus* (MRSA) isolates. This study extended to isolate six bacteriophages of different plaques morphology and size targeting these multidrug resistant bacteria. The isolated phages showed variations in their abilities to infect and lysis the target pathogen. These phages were selected for characterization. Bacteriophages active against *Salmonella* serovars named (Salmacey1, Salmacey2, and Salmacey3), *Proteus* (Protmacey), *S. aureus* (Staphmacey), and *Bacillus*

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(Bacmacey). The electron micrographs of negatively stained preparations of these phages revealed that they belong to *Myoviridae, Podoviridae, Siphoviridae* families. The results of host range assay revealed that these *Salmonella* bacteriophages were polyvalent and thus capable of infecting different strains of *Salmonella* serovars, *Citrobacter freundii ,Enterobacter and E.coli*. Bateriophages (Protmacey, Staphmacey, Bacmacey) were restricted to only their specific hosts. Only the isolated bacteriophages were thermostable, inbetween temperature ranges of 30–70°C, and the activity of isolated phages was rapidly decreased toward acidity compared to pH7. The one step growth curve for these phages were determined and the results of burst sizes and latent periods were determined. Molecular analyses indicated that these phages contained double-stranded DNA genomes. The lytic activities of the phages against the most multidrug resistant serovars *S. Kentucky, Proteus sp., Staph. aureus* and *B.cereus* as host strain was evaluated. The results showed that these bacteriophages were able to completely kill the growth of tested bacteria *in vitro*. These results suggest that phages have a high potential for phage application to control *Salmonella* serovars , *Proteus sp., Staph. aureus* and *B.cereus* isolated from broilers in Egypt.

Keywords: Food borne pathogens, broilers, multi drug resistant, phages

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