



Faculty of Medicine and Health Sciences

**Isolation and Characterisation of Bacteriophages infecting
Klebsiella pneumoniae from Sewage Samples in Sarawak, Malaysia**

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Isolation and Characterisation of Bacteriophages infecting
Klebsiella pneumoniae from Sewage Samples in Sarawak, Malaysia

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.



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Jeremiah 29:11

“For I know the plans I have for you,” declares the Lord, “plans to prosper you and not to harm you, plans to give you hope and a future.

ABSTRACT

A group of nosocomial multidrug-resistant pathogens that posed threats to immunocompromised patients have previously been referred to by the acronym ESKAPE. The ESKAPE pathogens which include Gram-negative *Klebsiella pneumoniae* are considered a great threat, due to the emergence of strains that are resistant to all or most available antibiotics. Misuse and extensive consumption of broad-spectrum antibiotics in hospitalised patients have allowed the evolution of drug-resistant bacterial strains by producing defense mechanisms such as extended-spectrum β -lactamases and diverse aminoglycoside-inactivating enzymes. Many of these strains are highly virulent and exhibit a strong tendency to propagate. In this study, five lytic *Klebsiella* bacteriophages namely ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10, and ϕ KPaV12 were isolated from domestic sewage at Universiti Malaysia Sarawak (UNIMAS) and characterised based on their biological properties, including bacteriophage morphology, host range, growth curve, bacteriophage multiplicity of infection (MOI) and structural protein composition. These bacteriophages have large burst size with high titer assay between $10^8 - 10^{12}$ pfu / mL and were predominantly stable under 4 °C. Two among the five bacteriophages were capable of efficiently lysing more than five *Klebsiella pneumoniae* strains out of 18 clinical and community-acquired isolates from Borneo Medical Centre (BMC) and students of UNIMAS, respectively. These bacteriophages exhibit several properties indicative of potential utility in phage cocktails and phage-antibiotic synergy (PAS) approach.

Keywords: *Klebsiella pneumoniae*, multidrug-resistant, sewage samples, bacteriophages, phage approaches

Pengasingan dan Pengenalpastian Bakteriofaj menjangkiti Klebsiella pneumoniae dari Sampel Kumbahan di Sarawak, Malaysia

ABSTRAK

Sekumpulan patogen nosokomial yang resisten terhadap pelbagai jenis antibiotik telah menimbulkan ancaman kepada pesakit yang lemah daya tahan. Patogen nosokomial ini telah dirujuk sebagai akronim ESKAPE. Patogen ESKAPE, termasuklah bakteria Gram-negatif Klebsiella pneumoniae yang dianggap sebagai ancaman besar, kerana resisten dengan kebanyakan antibiotik yang sedia ada. Penyalahgunaan antibiotik spektrum luas oleh pesakit-pesakit telah menyebabkan evolusi bakteria yang resisten dengan pelbagai jenis antibiotik melalui mekanisme pertahanan seperti spektrum lanjutan β -laktamase dan enzim yang menyah-aktif aminoglikosida. Kebanyakan strain bakteria Klebsiella pneumoniae menunjukkan daya virulen yang tinggi. Kajian ini telah berjaya memencilkan lima bakteriofaj Klebsiella iaitu ϕ KPaV03, ϕ KPaV04, ϕ KPaV08, ϕ KPaV10, dan ϕ KPaV12 dari sampel kumbahan domestik Universiti Malaysia Sarawak (UNIMAS) dan dikategorikan berdasarkan sifat biologi, termasuk morfologi bakteriofaj, kesan jangkitan bakteriofaj terhadap pelbagai jenis Klebsiella pneumoniae, lengkung pertumbuhan bakteriofaj, pergandaan jangkitan bakteriofaj (MOI) dan komposisi struktur protein. Kesemua bakteriofaj yang terpilih mempunyai saiz letusan besar dengan kadar titer yang tinggi antara 10^8 - 10^{12} pfu / mL dan kebanyakannya stabil di bawah suhu 4 °C. Dua daripada lima bakteriofaj mampu membasmi lebih daripada lima strain Klebsiella pneumoniae daripada 18 isolat klinikal dan komuniti dari Pusat Perubatan Borneo (BMC) dan pelajar-pelajar

UNIMAS. Disamping itu, bakteriofaj ini juga telah mempamerkan beberapa sifat yang menunjukkan potensi kegunaan dalam koktel bakteriofaj dan juga secara pendekatan sinergi bakteriofaj-antibiotik (PAS).

Kata kunci: *Klebsiella pneumoniae, resisten pelbagai antibiotik, sampel kumbahan, bakteriofaj, pendekatan bakteriofaj*

TABLE OF CONTENTS

	Page
DECLARATION	i
ACKNOWLEDGEMENT	ii
ABSTRACT	iii
<i>ABSTRAK</i>	iv
TABLE OF CONTENTS	vi
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvii
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW	1
1.1 General Introduction	1
1.2 The History of Therapeutic Bacteriophages	3
1.3 Emergence of Antibiotic-Resistant Bacteria	4
1.4 <i>Klebsiella pneumoniae</i> in a Healthcare Setting	5
1.5 Antibiotic Resistance Mechanism of <i>Klebsiella pneumoniae</i>	7
1.5.1 Prevalence of Antibiotic-Resistant <i>Klebsiella pneumoniae</i> in Malaysia	9
1.6 Bacteriophage Therapy	10
1.6.1 Type of Bacteriophages	10
1.6.2 Bacteriophages Life Cycle	12

1.6.3	Pros and Cons of Phages as Antimicrobials	14
1.6.4	Potentials, Safety and Current Clinical Trials of Therapeutic Bacteriophages	16
1.6.5	Isolated Bacteriophages Infecting <i>Klebsiella pneumoniae</i>	18
1.7	Study Objectives	20
CHAPTER 2: MATERIALS AND METHODS		21
2.1	Culture Medium Sterilisation	21
2.1.1	Filter Sterilisation	21
2.1.2	Heat Sterilisation	21
2.2	Culture Media and Reagents Preparation	21
2.2.1	Preparation of Media Broth – Luria-Bertani (LB) Broth	21
2.2.2	Preparation of Media Agar – Luria-Bertani (LB) Agar	21
2.2.3	Preparation of Media Agar – Mueller-Hinton (MH) Agar	22
2.2.4	Preparation of Glycerol Solution (50 % v/v)	22
2.2.5	Preparation of SM Buffer	22
2.2.6	Preparation of Phosphate Buffered Saline (PBS) Buffer	22
2.2.7	Preparation of 1X Tris-Acetate EDTA (TAE) Buffer	23
2.2.8	Preparation of 0.1M Calcium Chloride (CaCl ₂) Solution	23
2.3	Antibiotic used for Antibiotic Sensitivity Testing (AST)	23
2.4	Bacterial Strains	24
2.5	Preparation of <i>Klebsiella</i> spp. Log-phase Culture Broth	25

2.6	Verification of <i>Klebsiella pneumoniae</i> Genomic DNA	26
2.6.1	Wizard® Genomic DNA Purification Kit by Promega	26
2.6.2	Bacterial 16S rRNA PCR primers	26
2.6.3	Bacterial 16S rRNA PCR Condition	27
2.6.4	Agarose Gel Electrophoresis	27
2.6.5	Bacterial 16S rRNA Sequencing	28
2.7	Antibiotic Sensitivity Testing on <i>Klebsiella pneumoniae</i> Community-acquired Isolates	28
2.8	Housekeeping Genes Sequencing of <i>Klebsiella pneumoniae</i>	28
2.8.1	Housekeeping PCR primers	29
2.9	Bacteriophages Isolation, Purification, and Titration	30
2.9.1	Bacteriophages Sample Collection	30
2.9.2	Bacteriophages Enrichment	31
2.9.3	Bacteriophages Serial Dilution	32
2.9.4	Plaque Assay – Double-layer Agar Overlay Technique	32
2.9.5	Identifying and Verifying Putative Plaques (Spot Assay)	32
2.9.6	Bacteriophage Titration	33
2.10	Bacteriophages Characterisation	33
2.10.1	Phage Host Range Analysis	33
2.10.2	Step-by-Step (SBS) Phage Isolation Method	34

2.10.3	Single-step Growth Curve	34
2.10.4	Multiplicity of Infection (MOI)	35
2.10.5	High Titer Stock of Purified Bacteriophages	35
2.10.6	Bacteriophage DNA Extraction	35
2.10.7	Characterisation of Genes Encoding Bacteriophage Proteins	36
2.11	Phage Cocktail and Phage Antibiotic Synergistic against Antibiotic-Resistant <i>Klebsiella pneumoniae</i>	39
2.11.1	Phage Cocktail	39
2.11.2	Minimum Inhibitory Concentration (MIC) Selection	40
2.11.3	Phage-Antibiotic Synergy (PAS)	41
CHAPTER 3: RESULTS		43
3.1	Verification of <i>Klebsiella pneumoniae</i> Genomic DNA	43
3.1.1	DNA Fragments Visualisation by Agarose Gel Electrophoresis	43
3.1.2	NCBI BLAST® Analysis	44
3.2	Determination on <i>Klebsiella</i> spp. Log-phase	45
3.3	Antibiotic Sensitivity Testing (AST) on <i>Klebsiella pneumoniae</i> Clinical Isolates	46
3.4	Housekeeping Genes Sequencing Analysis of <i>Klebsiella pneumoniae</i>	49
3.5	Bacteriophages Isolation, Purification and Titration	54
3.5.1	Bacteriophages Serial Dilution	54
3.5.2	Step-by-Step (SBS) Method	55

3.5.3	Bacteriophage Host Range Analysis	56
3.5.4	Bacteriophage Titer	59
3.6	Bacteriophage Characterisation	59
3.6.1	Plaque Morphology	59
3.6.2	Multiplicity of Infection (MOI)	61
3.6.3	Single-Step Growth Curve	61
3.6.4	Bacteriophages Structural Protein Extraction	64
3.6.5	Bacteriophage Structural Protein Sequencing Analysis by UniProt Blast	68
3.7	Phage Cocktail and Phage-Antibiotic Synergy Approaches against Antibiotic Resistant <i>Klebsiella pneumoniae</i>	69
3.7.1	Phage Cocktail	69
3.7.2	Minimum Inhibitory Concentration (MIC) Selection	70
3.7.3	Comparison of Phage Cocktail and Phage-Antibiotic Synergy Approaches	71
CHAPTER 4: DISCUSSION		75
4.1	Verification of <i>Klebsiella pneumoniae</i>	75
4.2	Housekeeping Genes Sequencing Analysis	76
4.3	Antibiotic Sensitivity Test	78
4.4	Bacteriophage Isolation	79
4.5	Bacteriophages Plaque Morphologies	80
4.6	Host Range	80

4.7	Single-step Phage Growth Curve	81
4.8	Bacteriophage Structural Proteins Analysis	82
4.9	Minimum Inhibitory Concentration (MIC)	83
4.10	Phage Cocktail	84
4.11	Phage-Antibiotic Synergy (PAS) Approaches	85
	CHAPTER 5: CONCLUSION AND FUTURE STUDIES	87
	REFERENCES	89
	APPENDICES	105

LIST OF TABLES

		Page
Table 2.1	List of antimicrobial agents used against <i>Klebsiella pneumoniae</i>	23
Table 2.2	<i>Klebsiella</i> spp. isolates used throughout the experiment	24
Table 2.3	Components of <i>Klebsiella pneumoniae</i> 16S rRNA PCR mixture in 50 μ L reaction	27
Table 2.4	Universal sequencing primers for <i>Klebsiella pneumoniae</i> housekeeping genes	29
Table 2.5	Components of <i>Klebsiella pneumoniae</i> housekeeping genes PCR mixture in 25 μ L reaction	30
Table 2.6	PCR cycle condition for 25 μ L of <i>Klebsiella pneumoniae</i> housekeeping genes	30
Table 2.7	Specific primers used for Phage Structural Protein analysis	37
Table 2.8	Components of <i>Klebsiella</i> phage structural proteins PCR mixture in 30 μ L reaction	37
Table 2.9	PCR condition for Lysin gene	38
Table 2.10	PCR condition for Viral Capsid Assembly protein gene (g20)	38
Table 2.11	PCR condition for Major Capsid protein gene (g23)	38
Table 2.12	PCR condition for Tail Fiber protein gene (T1)	39

Table 3.1	The percentage of BLAST similarity of <i>Klebsiella</i> spp. 16S rRNA PCR sequences	45
Table 3.2	Antibiotics sensitivity testing of <i>Klebsiella pneumoniae</i> clinical strains (CRKP1 to KP9)	48
Table 3.3	Antibiotics sensitivity testing of <i>Klebsiella pneumoniae</i> clinical and non-clinical strains (KP10 to ATCC KP)	49
Table 3.4	Sequence query of <i>Klebsiella</i> locus under MLST scheme	50
Table 3.5	Bacteriophages isolated using different host strains respectively by SBS method	56
Table 3.6	Assessment of phages host range by spot assay	58
Table 3.7	Average titer (pfu / mL) of phage isolates	59
Table 3.8	Plaque assay analysis of <i>Klebsiella pneumoniae</i> clinical strains infected with increasing doses of isolated <i>Klebsiella</i> phages dilution (MOI)	61
Table 3.9	Summary of amplification of each isolated phage structural protein genes	64
Table 3.10	Characteristics of sequenced <i>Klebsiella</i> phages	68
Table 3.11	Minimum inhibitory concentration (MIC) of Gentamicin-resistant <i>Klebsiella pneumoniae</i> strains	71

LIST OF FIGURES

	Page	
Figure 1.1	Electron microscope images of three families of tailed phages	11
Figure 1.2	The actions of lytic phages as bacterial predators achieve several steps to attain host destruction	13
Figure 2.1	Location of sampling site in Sarawak with GPS coordinates	31
Figure 3.1	Agarose gel electrophoresis of PCR product for universal primers 27F and 152R	43
Figure 3.2	Agarose gel electrophoresis of PCR product for universal primers 27F and 152R	44
Figure 3.3	Growth curve of ATCC KP within 24 h using OD600 measurements. Each point corresponds to the average of three determinations \pm standard deviation	46
Figure 3.4	Antibiotic sensitivity test - Different antibiotics tested on KP15	47
Figure 3.5	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> rpoB gene using Mega 6 software	51
Figure 3.6	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> gapA gene using Mega 6 software	52
Figure 3.7	Phylogenetic tree resulting from Maximum Likelihood Method for <i>Klebsiella pneumoniae</i> tonB gene using Mega 6 software	53

Figure 3.8	Three different phage plaque morphologies (A, B and C) infecting host KP17	55
Figure 3.9	Spot assay of different phages spotted on bacterial host KP10	57
Figure 3.10	Different plaque morphologies formed by isolated phages infecting <i>Klebsiella pneumoniae</i> strains from sewage influent	60
Figure 3.11	The single-step growth curve assay for phage ϕ KPaV03 infecting ATCC KP at MOI 1. The latent period takes less than 20 mins.	62
Figure 3.12	The single-step growth curve assay for phage ϕ KPaV04 infecting CRKP1 at MOI 10. The latent period takes approximately 60 mins.	62
Figure 3.13	The one-step growth curve assay for phage ϕ KPaV08 infecting KP17 at MOI 0.01. The latent period takes less than 5 mins.	63
Figure 3.14	The one-step growth curve assay for phage ϕ KPaV10 infecting ATCC KP at MOI 0.1. The latent period takes approximately 20 mins.	63
Figure 3.15	The one-step growth curve assay for phage ϕ KPaV12 infecting KP15 at MOI 0.01. The latent period takes less than 10 mins.	64
Figure 3.16	PCR products of Lysin gene (Lys)	65
Figure 3.17	PCR products of Major Capsid Protein gene (g23)	66
Figure 3.18	PCR products of Tail Fiber gene (T1)	67
Figure 3.19	Phage cocktail approach against single <i>Klebsiella</i> spp. strains	69

Figure 3.20	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ATCC KP	72
Figure 3.21	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus CRKP1	72
Figure 3.22	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP2	73
Figure 3.23	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus ESKP3	73
Figure 3.24	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP5	74
Figure 3.25	Time-kill curves of phage cocktail (PC), phage-antibiotic synergy when treated with sub-inhibitory concentration of Gentamicin (PA) and growth control curve (GC) versus KP9	74

LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
blaKPC	<i>Klebsiella pneumoniae</i> Carbapenemase
CAP	Community-acquired Pathogens
CO ₂	Carbon Dioxide
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded Deoxyribonucleic Acid
g	Gram
ICTV	International Committee on Taxonomy of Viruses
kb	Kilobase
LB	Luria-Bertani
MDR	Multi-drug Resistant
mL	Milliliter
mm	Millimeter
mg	Milligram
MOI	Multiplicity of Infection
NDM	New Delhi Metallo- β -Lactamase
nm	Nanometer
OD	Optical Density
RNA	Ribonucleic Acid
rpm	Rotation per Minute
μ mol	Picomole
PCR	Polymerase Chain Reaction

PFU	Plaques per Unit
ssDNA	Single-stranded Deoxyribonucleic Acid
ssRNA	Single-stranded Ribonucleic Acid
TAE	Tris-Acetate-EDTA
TEM	Transmission Electron Microscope
V	Voltage
v/v	Volume per Volume
μL	Microlitre

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

The bacteriophages were independently discovered by British microbiologist Frederick Twort in 1915 and French-Canadian microbiologist Felix d'Hérelle in 1917 (Carlton, 1999). A discovery which occurred about 20 years before the practical application of penicillin, the first antibiotic (Matsuzaki et al., 2005). At the time of discovery, bacteriophages were regarded as a potential treatment for bacterial infections and have been developed to control bacterial diseases such as dysentery, cholera, and gangrene (Dublanquet & Bourne, 2007).

Regardless of the attributes and unique properties of bacteriophages being able to fight bacterial infections, it was then soon abandoned in the 1940s by the West with the arrival of the antibiotic era (Matsuzaki et al., 2005). With the rise of pharmaceutical antibiotics in the mid-20th century, along with a better understanding of diseases and sanitation, both quality of life and life expectancy in the industrialized world drastically improved (Lin et al., 2017). Antibiotics rapidly became an indispensable medical tool with millions of kilograms used globally each year in the prophylaxis and treatment of people, animal, and agriculture (Levy & Bonnie, 2004).

However, the excessive usage and prescription of antibiotics leads to the emergence of pathogenic bacteria resistant to multiple antimicrobial agents such as the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) (Weber-

Dabrowska et al., 2016). Antibiotic-resistant genes encoding for resistance to common antibiotics, including β -lactams, aminoglycosides, and tetracycline, are posing a major threat to current medical treatment of common diseases, and these genes now appear to be abundant in the environment (Lin et al., 2017).

The growing of multidrug-resistant pathogens threatens to make the revolutionary achievements of modern medicine obsolete, posing a concern of re-entering the “pre-antibiotics” era (Jeney, 2012). For example, Lin et al., (2017) mentioned the lack of treatment options for hospital-acquired carbapenem-resistant *K. pneumoniae* infections caused a 40 % - 50 % mortality rate in the United States beginning of 2000. Carbapenem resistance in *K. pneumoniae*, mainly attributed to the production of *K. pneumoniae* carbapenemase (KPC) enzyme which is able to destroy carbapenems and causes resistance against a wide spectrum of antibiotics.

Rekindling phage therapy can be a good option for solving the problem of multidrug-resistant pathogens such as *K. pneumoniae*. Major advantages of phage therapy compared to conventional antibiotic treatment are such that phages are highly specific and easy to obtain. Thus, it can be used in cases of sudden bacterial disease outbreaks. Moreover, they do not have chemical side effects like antibiotics (Baharuddin et al., 2017).

Therefore, the objectives of this study were to isolate and characterise lytic bacteriophages with therapeutic potential against clinical- and community-acquired *K. pneumoniae* strains. It is hoped that this study will be able to contribute to future phage therapy studies and applications.

1.2 The History of Therapeutic Bacteriophages

Bacteriophage, or simply called as phages, are viruses that specifically infect and lyse bacteria. The term bacteriophage was coined by Felix d'Herelle in 1916, who derived it from the word 'bacteria' and a Greek word 'phagein' which means 'to devour'. Phages, like all other viruses, are absolute parasites as they have no machinery to generate energy and ribosomes for making proteins even though they carry all the information to direct their own reproduction in an appropriate host. (Kutter & Sulakvelidze, 2005).

In 1910, d'Herelle first observed the bacteriophage phenomenon while studying microbiologic means of controlling an epizootic locusts in Mexico which he then applied his knowledge on phage to treat the dysentery outbreak among several French soldiers (Sulakvelidze, Alavidze, & Morris Jr., 2001). Following the success, the usage of bacteriophage as a therapeutic agent to treat bacterial infections has been widely applied circa the 1930s and 1940s in Eastern Europe and the former Soviet Union. However, it was abandoned by Western Medicine in the 1940s after the discovery of penicillin which soon became widely available (Matsuzaki et al., 2005).

The reason for abandoning the therapeutic usage of phages in the West was due to the lack of appropriate controls and inconsistent results (Wittebole et al., 2014). Moreover, at that time, the biological viral nature and mechanism of phage has yet to be determined and thoroughly studied until they were visualised in the 1940s after the invention of electron microscopy (Cisek et al., 2017).

Merril et. al., (2003) stated that the applications of phage as practiced in the Soviet Union including Poland have been extensively evaluated in which according to one of the review papers from 1998, only 27 papers dealing with bacteriophage therapy were published

between 1966 and 1996. Nineteen of these were from laboratories in Poland and Russia, where research on bacteriophage therapy had never dimmed, and where patients infected with *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Shigella*, and *Salmonella* were reportedly treated with 80 to 85 percent success (Ho, 2001). Likewise, Bull et al., (2002) has reviewed on the earlier experimental work on phage therapy and prophylaxis and mentioned several impressive studies using phage to treat and prevent bacterial infections in animals including work done by Williams S. H. and team in 1987.

However, details such as the primary qualitative data such as phage dosages and clinical criteria were not properly reported and documented. Hence, most of the studies from Eastern Europe are unable to meet the present standards for pharmaceutical approval in countries that require certification based on the results of efficacy and pharmacokinetic studies in animals and humans.

1.3 Emergence of Antibiotic-Resistant Bacteria

Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics were successfully used to treat bacterial infections in humans and animals, as well as in agriculture. However, the effectiveness of antibiotics is challenged by the increasing number of antibiotic-resistant bacteria. Most of the available antibiotics, including β -lactams, are becoming less effective and in some cases, resistance rates exceed 98 % (Akinkunmi et al., 2014). For instance, El-Shibiny & El-Sahhar (2017) have reported on few multidrug-resistant strains such as *Escherichia coli* O104:H4 and some *Salmonella* isolates from poultry which have been found to be resistant to at least 14 different antibiotics, and about 90 % of the *Salmonella* isolates were found to be resistant to at least one or more antibiotics tested.