Investigation of virulence factors of carbapenem resistant *Klebsiella pneumoniae* isolates and identification of vB_KpnS_Kp13 bacteriophage

Doctoral (Ph.D.) Thesis

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2020

I. INTRODUCTION

Klebsiella pneumoniae is an opportunistic, Gram-negative, rod-shaped bacterium belonging to the family *Enterobacteriaceae*. It is present in the respiratory tract and feces of about 5% of normal individuals. This species is the most medically important species of the family *Enterobacteriaceae* after *Escherichia coli*.

K. pneumoniae is an important hospital-acquired pathogen (nosocomial pathogen) that is a frequent aetiological agent of septicemia, wound and blood infection, urinary tract infection, intra-abdominal infections and pneumonia in immunocompromised individuals. It is also an important pathogen with respect to community-acquired infectious diseases, such as community-acquired pneumonia. *K. pneumoniae* isolates possess a variety of virulence factors that facilitate infection and survival in the host. Podschun and Ullmann (1998) documented that the virulence factors of *K. pneumoniae* consist of four major bacterial factors: (1) capsule, for inhibiting of phagocytosis; (2) lipopolysaccharides (LPS), for avoidance of host serum complement factors; (3) fimbriae, for adhesion and biofilm formation; and (4) siderophores, for iron acquisition.

Klebsiella spp. is genetically heterogeneous and strains within species and subspecies can be discriminated by a number of methods including biochemical tests, analysis of antigenic specificities, bacteriophage susceptibility typing, bacteriocin susceptibility or production typing and molecular typing methods. Five different phenotypic tests are mostly used to characterize isolates, like expression of type I and type III fimbriae, biofilm formation capacity, siderophore production, serum resistance and hypermucoviscosity (HMV). The HMV-phenotype of certain serotypes (K1 and K2), where the polysaccharide network shows an extreme stickiness, is associated with high pathogenicity of *K. pneumoniae*. Molecular typing methods include two types: protein based and nucleic acid-based methods. Molecular typing methods aim to discriminate strains based on the differences in their genomic DNA sequence and organization. Over the last years many molecular typing methods have been applied to investigate outbreaks due to *Klebsiella* strains and to compare strains from different time points and geographic origins. The most common molecular typing methods are: plasmid profile analysis, polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE), and whole-genome sequencing.

K. pneumoniae strains harbouring extended spectrum β-lactamases (ESBL) and metallo-β-lactamases, conferring resistance to many of antibiotics available and limiting treatment options, have been described in many parts of the world. These resistant pathogens are considered clinically important because they cause nosocomial infections that commonly appear in outbreaks.

Antimicrobial resistance is commonly related to the spread of transmissible plasmids and the acquisition of resistance genes that normally occur by horizontal gene transfer, which may also carry virulence determinants. For pathogen survival, the acquisition of resistance and virulence traits are necessary, and some reports suggest that they may have an essential role in the pathogenesis of *K. pneumoniae* infections. Approximately 80% of nosocomial infections are caused by multidrug-resistant (MDR) strains of *K. pneumoniae* strains. Since 1980, third generation cephalosporins, e.g. cefotaxime, ceftazidime and ceftriaxone, have been introduced as useful and effective drugs against most nosocomial infections. However, the excessive use of cephalosporins in clinical practice has resulted in increased bacterial resistance to these antibiotics, especially in *Enterobacteriaceae* family. Resistance to β-lactam antibiotics among the *Enterobacteriaceae* family is a result of the expression of ESBL genes. Among the members of *Enterobacteriaceae*, *K. pneumoniae* has the highest level of ESBLs. Several studies have shown that the most prevalent ESBL-producing bacterium in Asia, Europe and North America is *K. pneumoniae*. ESBLs arise due to mutations in β-lactamase genes. ESBL-producing *K. pneumoniae* are usually resistant to common antibiotics. Therefore, treatment for these infections are limited. Recently, researchers have been searching for alternative methods to control these infections, including combination therapy with alternative options, e.g. bacteriophages, essential oils and nanoparticles, in an attempt to reduce the incidence of antibiotic resistance.

Bacteriophages (or phages) are viruses that infect bacteria. Effects of phages were first reported in 1859 by Ernest Hankin, who observed the bactericidal effects of filtered water from the river Ganges, against *Vibrio cholera* isolates. However, the discovery of phages is usually credited to two other researchers, Frederick Twort (1915) and Felix D'Herelle (1917), who independently described phages as filterable, transmissible agents capable of bacterial lysis. D'Herelle immediately realized the potential for phages to be used as therapeutic agents and became the first proponent of phage therapy.

Phages are the most ubiquitous organism on Earth, with estimates of their total population reaching 10^{31}. The International Committee on Taxonomy of Viruses (ICTV) conventions group viruses sharing characteristics together in orders, which can be broken down into families. These families can be further broken down into genera, based on genome configuration and size. More than 90% of the known phages are tailed and belong to the order *Caudovirales*. These phages possess double-stranded DNA (dsDNA) genomes and are further divided into families based on their tail length. The *Myoviridae* family comprise phages with long, contractile tails; the *Podoviridae* family comprise phages with very short tails; and the *Siphoviridae* family comprise those with long, non-contractile tails (Fig. 1). These phages possess icosahedral heads made of

protein which encapsulates the DNA. Most dsDNA phages have genomes larger than 15 kbp due to the virion structure and assembly genes. The *Myoviridae* typically have larger genomes (>125 kbp), while *Siphoviridae* have genomes longer than 20 kbp.

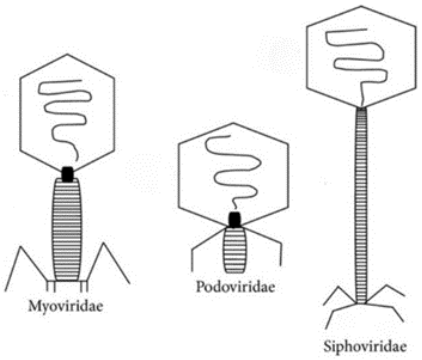

Figure 1. Morphology of the bacteriophages. The *Myoviridae* family comprise phages with long, contractile tails; the *Podoviridae* family comprise phages with very short tails; the *Siphovidae* family comprise phages with long, non-contractile tails (Source: thebacteriophages.org).

Based on their life cycles, bacteriophages can be divided into two major groups: lytic (lytic cycle of replication) and lysogenic (lytic and lysogenic cycles of replication). A third and less common group comprises the filamentous phages, which cause persistent infection of bacterial hosts without lysis or integration of genetic material into the host chromosome. The replication method is an important issue if practical application of phage is considered. The lytic cycle, which is also commonly referred to as the "reproductive cycle" of the bacteriophage, is a five-stage cycle, consisting of (1) adsorption, (2) penetration, (3) transcription and biosynthesis, (4) assembly and maturation, and (5) lysis. In the lysogenic cycle, the first two steps (adsorption and penetration) occur in the same way as in the lytic cycle. However, once the phage DNA is inside the cell, it is not immediately copied or expressed to make proteins. Instead, it recombines with a specific or random region of the bacterial chromosome. This causes the phage DNA to be integrated into the host chromosome.

Phage therapy is a unique method of treatment of bacterial infections using bacteriophages – viruses that specifically kill bacteria, including their antibiotic-resistant strains. Several studies show that phages could be used successfully for therapeutic purposes, both in humans and animals. Phages have been investigated as a potential means to eliminate pathogens like *Campylobacter jejuni* from raw food and *Listeria monocytogenes* from cheese. In agricultural practice phages were

used to fight against pathogens including *Campylobacter jejuni*, *Escherichia coli* and *Salmonella typhimurium* in farm animals, *Lactococcus garvieae* and *Vibrio anguillarum* pathogens from aquacultures, and *Erwinia amylovora* and *Xanthomonas juglandis* from plants of agricultural importance. The earliest application was, however, associated with human medicine. Phages have been used against diarrheal diseases caused by *Escherichia coli*, *Shigella flexneri*, *Shigella sonnei* or *Vibrio fetus* (*Campylobacter fetus*) and against wound infections caused by facultative pathogens of the skin, such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Phage cocktails for phage therapy are commonly created by combining a small number of known or newly isolated phages to create a therapeutic mixture with broader host specificity. Treatment with bacteriophages has numerous advantages over antibiotic therapy: (1) it is economic and effective against multidrug-resistant (MDR) bacteria, (2) it is selective, because it has high specificity for target bacteria, (3) its administration theoretically requires only a single dose of treatment, owing to its self-replicating nature. There are several factors however that influence the efficacy of phage infection. Through mutation, bacteria might resist adsorption by a particular phage as a result of: (1) alteration of the structure of the receptor site, (2) alteration of the exposure of the receptor site, (3) reduction of the density of the receptor sites.

Essential oils (EOs) are plant extracts that are rich sources of biologically active compounds, possessing antibacterial, antifungal, antiviral, insecticidal, antitumor and antioxidant properties. Although EOs are today widely used in aromatherapy, there has been an increased interest in their use as potential antimicrobials. This feature is mostly considered in food preservation and therapy. The increasing incidence of carbapenemase-producing *Klebsiella pneumoniae* strains (CP-Kps) in the last decade has become a serious global healthcare problem. Therapeutic options for the treatment of emerging hospital clones have drastically narrowed and therefore novel approaches must be considered.

Application of nanomaterials as antibacterial agents is another dynamically developing field of scientific research. Titanium-dioxide nanoparticles have shown antibacterial activity due to reaction of the TiO_2 surface with water. On exposure to UV irradiation, TiO_2 releases free radicals such as OH•, O_2•-, and H_2O_2. These potent oxidizing free radicals destroy bacterial membranes, enzymes and nucleic acids.

II. AIMS OF THE STUDY

A total of 39 *Klebsiella pneumoniae* isolates were collected from the Microbiology Laboratories of the University Hospital in Chester, England. The *K. pneumoniae* isolates collected between 2010 and 2017. The clinical strains were isolated from urine, blood culture, sputum and faeces.

Our first purpose was to investigate the comparative analysis of the *K. pneumoniae* isolates, therefore my study aimed to:

- Identification of *K. pneumoniae* strains from the different infections.
- Compare the virulence-associated features of the isolates in different phenotypic and genotypic tests.
- Reveal genetic relationship among the isolates.
- Determine the whole genome sequence of a representative strain.
- Compare the virulence potentials of 4 representative strains in different animal models.

The increasing incidence of carbapenemase-producing *K. pneumonaie* strains (CP-Kps) in the last decade has become a serious global healthcare problem. Therapeutic options for the treatment of emerging hospital clones have drastically narrowed, and for this reason novel approaches must be considered. For this reason, my research aimed to:

- Find a bacteriophage with lytic activity against the isolated *K. pneumoniae* strains.
- Determine phage morphology, host range and efficiency of plating (EOP) of the new phage.
- Determine the phage growth curve, killing efficacy and ability of biofilm degradation.
- Reveal the genomic organization of the new phage.
- Test the *in vivo* efficacy of the newly isolated phage in rescue experiments.
- Test the antimicrobial potentials of two alternative antimicrobial methods, essential oils (EOs) and titanium-dioxide nanoparticles, against one representative *K. pneumoniae* strain.

III. MATERIALS AND METHODS

In our experiments the following techniques were used in bacterial study:

- Biochemical tests and MALDI-TOF MS (matrix-assisted laser desorption/ionization-time of flight mass spectroscopy) analysis.
- Antibiotic profile tests (EUCAST guideline, 2018).
- Phenotypic tests:
    - agglutination assay,
    - biofilm assay,
    - siderophore production,
    - serum bactericidal activity,
    - hypermucoviscosity-test (HMV-test).
- Genotypic tests:
    - polymerase chain reaction (PCR),
    - plasmid isolation,
    - pulsed-field gel electrophoresis (PFGE).
- Cell invasion assay.
- Animal experiments (BALB/c mice were used):
    - lung infection (LI) model,
    - intraperitoneal (IP) infection model,
    - urinary tract infection (UTI) model,
    - gastrointestinal infection (GI) model.
- Whole genome sequencing and analysis of one representative *K. pneumoniae* strain.

In our experiments the following techniques were used in the phage study:

- Bacteriophage DNA isolation and restriction analysis.
- Transmission electron microscopy (TEM) analysis.
- Phage host range testing and efficiency of plating (EOP) analysis.
- One-step phage growth curve analysis.
- Time-kill activity screening.
- Biofilm degradation assay.
- Whole genome sequencing and phylogenetic analysis of phage.
- *In vivo* efficacy testing in mouse model.

Finally, in our experiments the following techniques were used for study of alternative antibacterial treatment:

- Study of the antibacterial activity of essential oils (EOs):
    - spot testing of the EOs on the lawn of *K. pneumoniae* 53/3 strain,
    - determination of minimal inhibitory concentration (MIC),
    - biofilm degradation assay.
- Study of the antibacterial activity of titanium-dioxide nanoparticles:
    - liquid culture screening.

IV. RERULTS AND DISCUSSION

IV.1. Virulence characteristics of *K. pneumoniae* isolates

A total of 39 isolates were collected from the Microbiology Laboratories of the University Hospital in Chester, England. Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Biochemical tests and MALDI-TOF MS analysis were used for controlling identification of *K. pneumoniae* isolates. All applied *K. pneumoniae* isolates were negative for indole test, methyl-red test, ornithine and arginine decarboxylase activity, and motility test. All applied isolates were positive for adonit fermentation, Voges-Proskaeur test, citrate, malonate, urease test and lysine decarboxylase activity. All isolates were positive for saccharose and lactose fermentation. Finally, we accomplished another identification of the clinical strains using the MALDI-TOF MS analysis.

Antibiotics susceptibility profile of the 39 isolates of *K. pneumoniae* was examined towards 17 different antibiotics using the disc diffusion method recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (2018). All isolates were resistant to penicillins, cephalosporins, fluoroquinolons and ertapenem from among the carbapenems. While 97.4% (38/39) of strains were resistant to tobramycin, 92.3% (36/39) of the strains were moderately sensitive to imipenem and meropenem. Furthermore, 69.2% (27/39) and 51.3% (20/39) of isolates were moderately sensitive to gentamicin and amikacin, respectively.

The phenotypic tests showed that, type I fimbriae were observed in ~95% (37/39) of the strains, consistent with results from the genotypic tests (*fimH-1*). Type I fimbriae were found to be essential for the ability of *K. pneumoniae* to associate with epithelial cell surfaces. Type III fimbriae was observed in ~87% (34/39) of the strains. These types of fimbriae allow adhesion to

various human tissue structures (kidney, lung, bladder) and are potent promoters of biofilm formation on biotic and abiotic surfaces. Therefore, they may have role in biofilm–associated infections (e.g. catheterized patients). Ninety-five percent (37/39) of the isolates were found to be positive for siderophore production. The first line of host defense against invading microorganisms includes the serum bactericidal activity, almost half of our strains (46%) were found to be resistant to this defense mechanism of the human body. All *K. pneumoniae* strains had a K24 capsular type, while only 10.3% (4/39) had the HMV-phenotype. The *rmpA* gene encoding a positive regulator of mucopolysaccharide expression was present in 4 strains, in agreement with the HMV-phenotype tests.

Macrorestriction profile analysis by pulsed-field gel electrophoresis (PFGE) was used to distinguish between 39 carbapenem-resistant *K. pneumoniae* isolates. Characteristic profiles were generated for each isolate by using the restriction endonuclease *XbaI*. Using a cutoff of 85% pattern similarity, the 39 *K. pneumoniae* isolates were grouped into 12 clusters.

The *K. pneumoniae* 53/3 genome measured 5 010 272 bp with a 51% G+C content (accession number SAMN15956199). It contains 5 671 predicted genes.

Virulence tests in the animal models and comparison the results to the hypervirulent *K. pneumoniae* reference strain NTUH-K2044 have revealed that all the tested isolates showed low level of virulence. The only exception was the *K. pneumoniae* 11/3 strain, that caused the death (on day 3) of all the experimental animals in the intraperitoneal (IP) tests, if the CFU was 4×10^7 per mouse when the bacterial challenge occurred. In the lung infection model, loss of body weight was detected 24 hours after the bacterial challenge. This result was the only indication that there was a slight difference in the virulence potential among the isolates. All tested *K. pneumoniae* strains (11/3, 50/1, 53/2 and 53/3) were able to evoke a mild urinary tract infection (UTI) in mice and in three cases (50/1, 53/2, 53/3), bacteria could be reisolated from the infected animals at the 10th day after infection. In the GI colonization model, all 4 strains transiently colonized the streptomycin-treated mouse intestine, but eventually became undetectable by the end of the third week.

The cell internalization assays performed on T24 bladder epithelial cells and INT407 intestinal cells revealed that although all strains had some degree of invasive capacity, this feature was considerably lower in the T24 bladder cell line.

IV.2. Identification of newly isolated lytic bacteriophage

The increasing incidence of carbapenemase-producing *K. pneumoniae* strains (CP-Kps) in the last decade has become a serious global healthcare problem. Therapeutic options for the treatment of emerging hospital clones have drastically narrowed, and for this reason novel approaches must be considered. Lytic phages, which kill their host following amplification and release of progeny phage into the environment, may offer an alternative strategy for combating bacterial infections.

Altogether 13 bacteriophages were isolated from the local wastewater (Pellérd) against *K. pneumoniae* strains. Four of them were effective against all *Klebsiella pneumoniae* strains possessing the K24 capsule. Based on their restriction profiles (*EcoRI* and *HindIII*) one proved to be distinct from the other 3 and this single one always showed a clear lytic zone (around 10 mm diameter) without the emergence of phage-resistant colonies and showed a characteristic wide halo (~ 4 mm) (Fig. 2). This phage was vB_KpnS_Kp13 and characterized in this study.

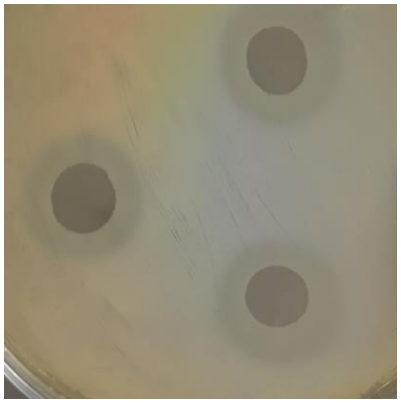

Figure 2. Plaque morphology of phage vB_KpnS_Kp13. Clear plaques (~10 mm) surrounded by a wide halo zone (~4 mm) formed by vB_KpnS_Kp13 with *K. pneumoniae* 53/3 lawn on double layer agar plate.

Transmission electron microscopy (TEM) analysis indicated that phage vB_KpnS_Kp13 comprised a ~60 nm diameter head and a flexible, non-contractile, ~200 nm tail (Fig. 3). Based on these traits, phage vB_KpnS_Kp13 was classified as *Caudovirales* in the *Siphoviridae* family.

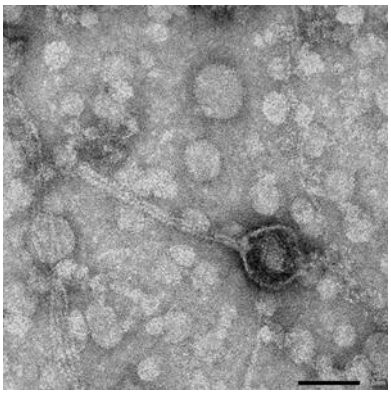

Figure 3. Electron micrograph of phage vB_KpnS_Kp13 shows the typical features of the *Siphoviridae* family. Phage was stained with 1.5 % w/v phospho-tungstic acid. Scale bar represents 50 nm.

Host range tests with 89 *Klebsiella spp.* isolates showed that phage vB_KpnS_Kp13 was only effective on *K. pneumoniae* strains with the K24 capsule, causing lysis in 100% (40/40) of these strains.

The one-step growth experiment was performed in order to determine the latent time period and burst size of the new phage particles. A triphasic curve was obtained showing the (1) latent period, (2) log or rise period, and (3) plateau period. The phage vB_KpnS_Kp13 showed relatively short latency period (18 min) followed by a rise period of 10 min and a growth plateau starting at 27 min. The burst size of vB_KpnS_Kp13 was ~220 phage particles per infected bacteria.

Phage vB_KpnS_Kp13 inhibited the proliferation of *K. pneumoniae* 53/3 in a concentration dependent manner. At multiplicity of infection (MOI) between 0.0001 and 100, phage vB_KpnS_Kp13 effectively inhibited the growth of *K. pneumoniae* 53/3 strain in LB broth for 24 h. Spot testing of the phage vB_KpnS_Kp13 on the lawn of different *K. pneumoniae* strains indicated that no resistant bacterium clones have emerged against the newly isolated phage.

The *K. pneumoniae* 53/3 strain is a strong biofilm producer (mean OD = 4.3 ± 0.2) based on the formerly established OD cut-off values (Vuotto et al., 2017). Here, pre-established biofilms were degraded by phage vB_KpnS_Kp13 in a MOI and time dependent manner. A 51.8 % loss in biomass was detected after 2 h incubation at MOI 10. Biomass reduction was further increased to 54.2 %, 57.5 %, and 72.9 % after 12, 24 and 48 h respectively, if compared to the OD values of the untreated biofilm control.

Biofilm degradation efficacy of phage vB_KpnS_Kp13 was also analysed with Confocal Laser Scanning Microscopy (CLSM). CLSM images showed that phage vB_KpnS_Kp13 firmly reduced the biomass and thickness of the biofilm architecture (Fig. 4).

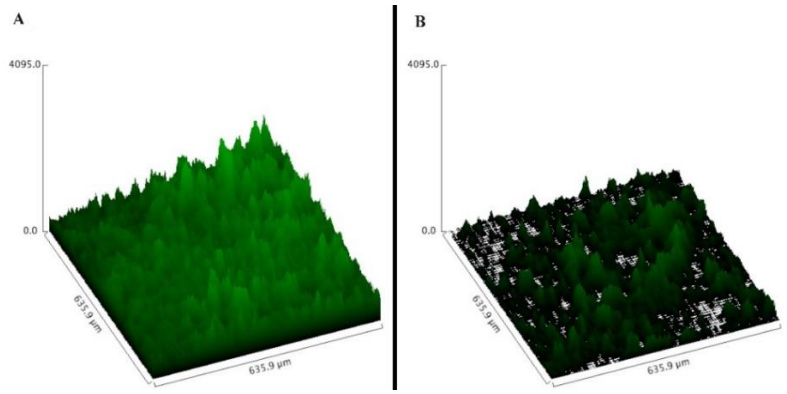

Figure 4. Visualization of *K. pneumoniae* 53/3 biofilm by CLSM. **A:** Control *K. pneumoniae* 53/3 biofilm surface plot. **B:** Phage vB_KpnS_Kp13-treated (2×10^9 PFU/ml) 53/3 biofilm surface plot. Surface plot was generated using CLSM microscopy software (Olympus FV1000).

The phage vB_KpnS_Kp13 genome measured 43,094 bp (accession number MK170446) with a 50.6% G+C content. It contains 75 predicted open reading frames (ORFs), while no tRNA gene was predicted by GeneMark program. The ORF analysis of the complete vB_KpnS_Kp13 genome revealed five major functional clusters: (1) DNA replication/modification/transcriptional regulations, (2) structure and packaging, (3) host lysis, (4) tail structure and (5) hypothetical or unknown functions.

A phylogenetic analysis of the complete vB_KpnS_Kp13 genome was performed using VICTOR web service. It was found that the *Klebsiella* JY917 phage is the closest relative of vB_KpnS_Kp13 with 90% similarity.

The therapeutic potential of vB_KpnS_Kp13 phage was revealed in an intraperitoneal (IP) mouse model, where the effect of vB_KpnS_Kp13 against *K. pneumoniae* 53/3 was strongly dependent on the time passed between the bacterial infection and phage administration. Survival of 100% of the mice, if treatment occurred 10 min after bacterial challenge, indicated the efficacy of vB_KpnS_Kp13 *in vivo*. Failure of the 1 h treatment suggested that *K. pneumoniae* 53/3 gained advantage and by this phage vB_KpnS_Kp13 treatment could only delay the death of the experimental animals.

IV.3. A potential role for alternative antimicrobial agents

In the present study, 13 essential oils were investigated for activity against *K. pneumoniae* 53/3 strain, using spot tests. We found that rosemary oil, peppermint oil, thyme oil, eucalyptus oil and two types of sage oil – *Salvia officinalis* and *Salvia sclarea* – had antibacterial and antibiofilm activity against *K. pneumoniae* 53/3 strain. The zone of inhibition above 6 mm in diameter was taken as a positive result. Minimum inhibitory concentration (MIC) for these 6 oils ranged from 0.3 to 0.5 µl/ml. Thyme oil showed maximum activity with MIC values. The results showed various percentages of reduction in biofilm formation of *K. pneumoniae* 53/3 strain. The EO of thyme exhibited strong antibiofilm activity against *K. pneumoniae* 53/3 strain (55.72% biofilm reduction in 24h).

As part of my study, we tested the antibacterial activity of different PF-titanium-dioxide nanoparticles against *K. pneumoniae* 53/3 strain. Our titanium-dioxide samples did not show any antibacterial effects under dark conditions, indicating that any possible release of P and/or F dopants do not affect the viability of the bacteria. PF-TiO_2 1h, 3h, 6h and 12h exhibited the highest activity under UV-A irradiation, where the living cell number dropped from 10^6 to 10^2 CFU/ml below 20 min, indicating the high antibacterial activity of these samples.

V. NOVEL FINDINGS OF THE THESIS

Our main findings can be summarized as follows:

1. We performed complex phenotypic and genotypic characterization of clinical *K. pneumoniae* isolates.
2. We proposed a new primer pair to genetic determinant of the K24 capsular type for *K. pneumoniae* strains.
3. We have detected the presence of *rmpA* gene in VIM-producing, carbapenemase-producing *K. pneumoniae* (CP-Kps) ST15 clone. To best of our knowledge this was not described before.
4. We confirmed the correlation between biofilm production and type III fimbriae.
5. Our results support the correlation between the presence of the *rmpA* gene and high mucopolysaccharides expression.
6. We have found that the *K. pneumoniae* 11/3 strain showed higher virulence in an animal IP model.
7. We identified a newly isolated lytic bacteriophage (vB_KpnS_Kp13) against a K24 capsular type, carbapenemase-producing *K. pneumoniae* strains.

8. We proved the antibacterial activity of thyme EO against *K. pneumoniae* 53/3 strain.
9. We have confirmed the photoinduced antibacterial activity of the differently modified PF-titanium-dioxide nanoparticles on *K. pneumoniae* 53/3 strain.

VI. ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisor, Dr. György Schneider for his help and for his continuous professional support.

I thank all my colleagues at Coutess of Chester Hospital for sharing with me their *K. pneumoniae* strain collections to work with.

I thank all the former and present members of the Institute of Medical Microbiology and Immunology. Special thanks to Dr. Zoltán Tigyi for his insightful comments and for sharing with me his precious bacterial strain collections. I thank Dr. Szilvia Zsóka Melegh for her help during the antibiotic profile tests. I would like to thank Prof. Dr. Levente Emődy who helped me to accomplish the animal study. I am thankful to my fellow PhD students, Bettina Schweitzer and Botond Pertics for inspiring me throughout my PhD studies. I am very grateful to Éva Molnár and Ildikó Denke for their priceless assistance with the experiments.

I thank Prof. Dr. Péter Putnoky for his help in performing the PFGE experiments and Dr. Ivelina Damjanova for her help in preparing the dendrogram and cluster analysis.

I am very grateful Dr. Tamás Kovács for assuring the whole genome sequencing. I thank Dr. Gábor Rákhely and Sarshad Koderivalappil for CLSM images. I also say thank Dr. László Kőrösi for providing us the titanium-dioxide nanoparticles.

Special thanks to Dr. Hajnalka Ábrahám for her support during my PhD work and Prof. Dr. László Seress for his help in the TEM analysis.

Last, but not least, I express my warmest thanks to my Mother for her love, support, advice and patience.

VII. LIST OF PUBLICATIONS

Articles related to thesis:

Horváth M., Kovács T., Koderivalappil S., Ábrahám H., Rákhely G., Schneider Gy.: Identification of a newly isolated bacteriophage against a K24 capsular type, carbepenem resistant *Klebsielle pneumoniae* belonging to the ST15 colnal lineage. *Scientific Reports*. 10: 5891. https://doi.org/10.1038/s41598-020-62691-8 (2020). (Impact factor: **4.116**)

Kőrösi L., Bognár B., **Horváth M.**, Schneider Gy., Kovács J., Scarpellini A., Castelli A., Colombo M., Prato M.: Hydrothermal evolution of PF-co-doped TiO_2 nanoparticles and their antibacterial activity against carbapenem-resistant *Klebsiella pneumoniae*. *Applied Catalysis B: Environmental*. Volume 231. p:115-122. https://doi.org/10.1016/j.apcatb.2018.03.012 (2018). (Impact factor: **14.229**)

Articles not related to thesis:

Schneider Gy., Szentes N., **Horváth M.**, Dorn Á., Cox A., Nagy G., Doffkay Zs., Maróti G., Rákhely G., Kovács T.: Kinetics of targeted phage rescue in a mouse model of systemic *Escherichia coli* K1. *Hindawi*. Article ID 7569645. https://doi.org/10.1155/2018/7569645 (2018). (Impact factor: **2.583**)

Kovács T., Lootz K., Dorn Á., Andrieu J., **Horváth M.**, Mátyás A., Schneider Gy.: Potential of small-scale jar system to extend the shelf life of raw meats, and hinder the proliferation of *Campylobacter jejuni* and *Enerohemorrhagic Escherichia coli*. *LWT – Food Science and Tecnology*. XXX. 1-9. http://dx.doi.org/10.1016/j.lwt.2016.10.058 (2016). (Impact factor: **2.7**)

Melegh Sz., Schneider Gy., **Horváth M.**, Jakab F., Emődy L., Tigyi Z.: Identification and characterization of CTX-M-15 producing *Klebsiella pneumoinae* clone ST101 in a Hungarian University Teaching Hospital. *Acta Microbiologyca et Immunologica Hungarica*. 63(3): 233-245. https://doi.org/10.1556/030.62.2015.3.2 (2015). (Impact factor: **0.568**)