Anti-MRSA – phage therapy alternatives for controlling MRSA

Key external stakeholders:
Pharmaceutical companies; bacteriophage-based therapeutics companies and research communities; public health agencies and health professionals; pig farmers and pig meat processors; veterinarians.

Practical implications for stakeholders:
- A single protein called LysK, from the bacteriophage staphylococcal phage K, can inhibit drug-resistant strains of Staphylococcus aureus.
- The protein has the ability to kill live methicillin resistant S. aureus (MRSA) and could be a strong candidate for commercialisation.

This discovery is important because antibiotic resistant S. aureus strains, in particular MRSA, are major causes of hospital related infections worldwide. The emergence and increasing incidence of these so called ‘superbugs’ combined with the absence of new antibiotics from the pharmaceutical sector demands that alternative anti-MRSA agents are evaluated and developed as a matter of urgency. The project exploited the use of bacteriophages, i.e. natural, specific anti-bacterial viruses, to eliminate antibiotic resistant S. aureus strains in biological environments.

Main results:
- Purified LysK protein is effective at eliminating MRSA in broth, cell culture, milk and blood.
- The phage eliminated S. aureus in the nostrils of mice.
- MRSA was not isolated from any of the pig herds tested to date and only a small percent (<2%) of personnel involved in the pig industry in Ireland are carriers of MRSA.

Opportunity / Benefit:
The opportunity exists to further investigate the potential of LysK as a potent pharmaceutical product against MRSA with a view to commercialisation. Expressions of interest from relevant companies are welcome.

Collaborating Institutions:
UL, UCD, Athlone IT, Cork IT, National MRSA Reference Lab - St. James Hospital
1. Project background:

*Staphylococcus aureus* is a major cause of infection in the human population causing a wide variety of conditions from simple abscesses to fatal sepsis. The continued use of antibiotics either in therapeutic or prophylactic applications for the control of *S. aureus* infections has major disadvantages, including the concomitant emergence of antibiotic-resistant strains. In particular, the emergence of antibiotic resistant clinical isolates of *S. aureus* such as the ‘Superbug’ MRSA is of increasing concern. Recent figures suggest that in Ireland in 2005, over 6000 hospital patients became infected with MRSA. This widespread development of resistance to frontline antibiotics poses a major threat to their use as therapeutics. As a result, efforts must now be directed to the development of new and alternative non-antibiotic treatments effective against drug-resistant *S. aureus*. Lytic bacteriophage (phage, i.e. natural, specific anti-bacterial viruses) kill bacteria via mechanisms that differ from those of antibiotics, and therefore, can be considered as antibacterials with a ‘novel mode of action’, a concept desired for all new antibacterial agents. Moreover, a number of recent studies have shown the enormous potential of the use of phage endolysins, rather than the intact phage, as potential therapeutics. During the phage life cycle, these enzymes specifically catalyse the breakdown of bacterial cell walls, thus allowing viral release from phage-infected cells. What makes endolysins attractive as potential therapeutics is their ability to cause ‘lysis from without’, i.e. they have the ability to degrade peptidoglycan and lyse their specific host even when applied externally. This project aimed to exploit anti-staphylococcal bacteriophages (phage, i.e.), for the elimination of MRSA and other drug resistant staphylococci in biological environments including blood. The endolysin from a previously-characterised broad host-range anti-staphylococcal bacteriophage K, LysK, was investigated for the biocontrol of multi-drug resistant *Staphylococcus aureus*.

2. Questions addressed by the project:

- Is LysK active against live cells of MRSA?
- Is the catalytic domain of LysK, CHAP$_K$, functional in the absence of the cell-wall binding domains?
- What are the properties and host range of CHAP$_K$?
- Is purified CHAP$_K$ active against live cells of MRSA?
- What is the prevalence of MRSA in the Irish pig population?

3. The experimental studies:

Over-expression and purification of LysK was one of the primary objectives of this project. To this end, the LysK protein was successfully cloned into a series of QIAexpress system vectors, *E. coli* expression vectors used to terminally His-tag the protein of interest. In addition, a method was developed to purify the over-expressed recombinant protein which included solubilisation and refolding of the expressed protein, followed by purification using a combination of nickel ion chromatography and ion exchange chromatography. X-ray crystallography screens by hanging drop were set up to optimise conditions for the crystal formation of LysK and its derivatives for 3D structure determination. A series of deletion mutants of the cloned LysK protein were created to undertake structure/function studies of LysK. The creation of these mutants was achieved through a combination of PCR, cloning and recombinant expression of truncated fragments of LysK in the QIAexpress vector system. Also, site directed
mutagenesis was performed with the the Quickchange II XL site-directed mutagenesis system to introduce amino acid substitutions at critical positions within the catalytic domains. Throughout the course of this project, a large bank of MRSA strains was collected, representing the main MRSA strains emerging on the island of Ireland. Multi-locus-sequence-typing (MLST) analysis was completed on all target MRSA strains. To investigate the effect of LysK at eliminating each of these targets, zymographic analysis was used. This technique is based on SDS-PAGE but includes an enzyme substrate co-polymerised with the polyacrylamide gel, for detection of the enzyme activity. In addition, a study was performed to evaluate the prevalence of MRSA in pigs and personnel involved in the pig industry in Ireland.

4. Main results:
The main findings of the project were as follows:

- The purified LysK protein proved to be effective at eliminating live MRSA cells. Highly active preparations of LysK were made and were used to eliminate cells in culture. In real-time, rapid cell clearing could be visualised both microscopically and in a test-tube of broth. Immediately after addition of LysK to cells on a slide, the disruption of MRSA cells under the light microscope is spectacular. These highly active preparations of LysK also effectively eliminate MRSA in blood and milk, with similar efficiencies to those seen in broth.

- The cysteine-histidine dependant amidohydrolase/peptidase domain from staphylococcal phage K (CHAPK) is one of two enzymatic domains from the endolysin (lysin) LysK. The CHAPK peptide was purified as a 165 amino acid molecule by cation exchange chromatography and its properties were established. It has a working pH range of 6 to 11, a temperature range from 5°C to 40°C and is effective in the presence salt concentrations of 0mM to 300mM.

- Site-directed mutagenesis of CHAPK revealed that the active site residues, Cys54, His117 and Asn136, were crucial for activity, in that their alteration eliminated lytic activity.

- The host range of CHAPK consists of all staphylococci including S. aureus, methicillin resistant S. aureus (MRSA), coagulase-negative (CoNS) and expolysaccharide (EPS) producing staphylococci. CHAPK also displayed activity against bacterial genera outside the host range of the native LysK (495 amino acid) protein. These included Gram-positive S. mutans, S. pneumoniae, S. thermophilis, S. pyogenes, M. luteus, M. lylae, M. flavus, N. halobia, A. agilis L. mesenteroides and C. maltaromanticum.

- MRSA was not isolated from any of the pig herds tested to date. Investigation of workers in pig-related professions demonstrated that carriage was extremely low, with less than 2% of the study population carrying MRSA in the anterior nares. The sequence types detected were ST22 and ST1307, both carrying SCCmec elements IV and II and both PVL-negative. The sequence type ST398, with high incidence in pigs in Europe, was not detected.

5. Opportunity/Benefit:
There is an opportunity to continue research on the potential of Lys K as an anti-MRSA treatment, with a view to commercializing it as a potent pharmaceutical product. Queries are welcome from companies interested in collaborating with the researchers, to consider funding mechanisms for commercialization purposes.

6. Dissemination:
Main publications:


**Popular publication:**


7. **Compiled by:** Dr. Olivia McAuliffe