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(54) Title: MEDICAMENTS

(57) Abstract: The present invention relates to medicaments suitable for use in the prevention and/or treatment of bacterial infection and/or colonisation. More particularly, the invention relates to medicaments comprising bacteriophages capable of binding to bacterial efflux pump components suitable for use in the prevention or treatment of diseases associated with Gram-negative bacteria. The invention also provides methods of treatment and methods of food preparation utilising such bacteriophages, as well as an assay by which such bacteriophages may be identified.



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MEDICAMENTS

The present invention relates to medicaments suitable for use in the prevention and/or treatment of bacterial infection and/or colonisation. More particularly, the invention relates to medicaments comprising bacteriophages capable of binding to bacterial efflux pump components suitable for use in the prevention or treatment of diseases associated with Gram-negative bacteria. The invention also provides methods of treatment and methods of food preparation utilising such bacteriophages, as well as an assay by which such bacteriophages may be identified.

Bacteria may be classified as either Gram-positive or Gram-negative based on their reaction to the Gram staining protocol. This protocol comprises the use of a crystal violet stain and safranin counterstain, the two staining steps being separated by an acetone-based decolourising step. Gram-negative bacteria are so named since they do not retain the Gram stain. Gram-negative bacteria are surrounded by a cell wall having a high lipid and low peptidoglycan content, and this cell wall composition allows the crystal violet stain to be washed away during decolourisation.

Gram-negative bacteria are frequently of medical or veterinary interest, since many Gram-negative species are pathogenic. The pathogenic nature of such bacteria is frequently associated with certain components of their cell walls, particularly the lipopolysaccharide (endotoxin) layer.

The class of Gram-negative bacteria encompasses many well-known bacterial genera including *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., *Haemophilus* spp., and *Moraxella* spp.

The various species of *Salmonella* are among the most medically relevant Gram-negative bacteria. Many species of *Salmonella* are capable of infecting humans, birds, reptiles and other animals. Two *Salmonella* serotypes, *S. Typhimurium* and *S. Enteritidis*, are

responsible for the majority of food poisoning incidents in humans. A further serotype of interest is S. Java, which has been linked with recent outbreaks in Scotland and 60% of the Dutch poultry flocks.

Salmonellosis is a major problem in most countries infecting over 160,000 individuals in the EU annually. Symptoms of salmonellosis include fever, headache, nausea, vomiting, abdominal pain and diarrhoea. Examples of foods involved in outbreaks of salmonellosis are eggs, poultry and other meats, raw milk and chocolate. Salmonella contamination of eggs was one of the main microbiological food safety issues of the 1990s. A Department of Health-funded survey in 1995/96 found that approximately 1 in 600 eggs were contaminated with Salmonella with the majority of contamination thought to be on rather than in the egg. In recent years a number of legislations have been passed to control Salmonella entry into the food chain.

The U.S. Centre for Disease Control and Prevention estimates that 75 million people suffer food-borne illnesses each year in the United States. These cases account for 325,000 hospitalizations and more than 5,000 deaths. The economic impact of such illnesses is high, costing an estimated \$5 to \$6 billion in direct medical expenses and lost productivity.

Infectious intestinal disease causes substantial morbidity and economic loss in the United Kingdom and is responsible for over 300 deaths and 35,000 hospital admissions annually in England and Wales.

In March 1989 new Salmonella legislation was announced, which covered compulsory slaughter and mandatory reporting of results of tests for Salmonella. Further legislation was introduced in 1993, with the release of the Poultry Breeding Flocks and Hatcheries Testing Order, which required that hatcheries can only supply flocks that are Salmonella enteritidis and typhimurium negative. Following the introduction of these legislations the contamination of poultry products was significantly reduced.

Salmonella legislation is supported by codes of practice and assurance schemes, which are industry led arrangements that have been developed to ensure that standards of welfare, traceability, husbandry, storage and other aspects of production are met in order to help improve customer confidence in the integrity of products. The Department for the Environment, Food and Rural Affairs (DEFRA) and the Food Standards Agency (FSA) support the development of assurance schemes concerning egg and poultry production as they provide consumers with assurance on food safety and can also provide producers with the opportunity for better marketing, subject to clearance from the competition authorities. Assurance schemes include assured chicken production and Lion Code of Practice.

The assured chicken production scheme is a voluntary industry funded programme to cover conditions of poultry production and slaughtering. Under the conditions of bird health all parent birds must be vaccinated with a licensed Salmonella Enteritidis and Salmonella Typhimurium vaccine.

The Lion Code of Practice is applied at each point in the egg production chain. All breeding flocks are tested weekly for Salmonella, with flocks testing positive for Salmonella Enteritidis and Salmonella Typhimurium being slaughtered. Within rearing farms all pullets are vaccinated against Salmonella Enteritidis. The lion code only requires compulsory vaccination against Salmonella Enteritidis, but does encourage vaccination against Salmonella Typhimurium.

From 2004 the European Zoonoses Directive will demand the slaughter of any flocks infected with a salmonella species of significance to human health, this directive exerts extreme pressure on breeders, rearers, layers and broilers to maintain salmonella free flocks. Consequently the demand for a vaccine that is effective against a number of Salmonella strains will be in significant demand.

In the light of the above it will be appreciated that there remains a well-recognised need for new medicaments and methods of treatment effective against Gram-negative bacteria. It is an object of certain aspects and embodiments of the present invention to provide

medicaments and methods of treatment for use in the prevention and/or reduction of bacterial colonisation and/or infection. It is an object of certain aspects and embodiments of the present invention to provide alternative medicaments and methods of treatment for use in the prevention and/or reduction of bacterial colonisation and/or infection. It is also an object of certain aspects and embodiments of the invention to provide methods of identifying bacteriophages susceptible to medical use.

In a first aspect of the present invention there is provided a medicament comprising a bacteriophage capable of binding to a bacterial efflux pump component. The inventors have found that medicaments in accordance with this aspect of the invention are effective in preventing and/or treating bacterial colonisation or infection of host animals. Medicaments in accordance with the invention may be used to eliminate harmful bacteria from an animal that would otherwise be subject to bacterial colonisation or infection.

Bacteriophages (often known simple as “phages”) are viruses that grow within bacteria. As they grow most bacteriophages kill their bacterial host during a process of bacterial cell lysis by which the next generation of bacteriophages, produced within the bacterial host, is released. These young phages then infect other nearby bacteria, eventually killing off the population of bacteria.

The ability of bacteriophages to kill bacterial hosts has been put to use in the treatment of diseases caused by bacterial infections. A series of reports on the use of the phage therapy on suppurative bacterial infections, such as empyemas, peritonitis and osteomyelitis, in humans have produced promising results. Most of the cases treated were chronic infections, and phage therapy was used as a last resort following the failure of all other forms of treatment.

Bacterial efflux pumps are involved in the excretion of substances harmful to bacteria from the bacterial cytoplasm to the extra-bacterial space. This excretion thus prevents or reduces the potentially damaging effects of the harmful substance. Bacterial efflux pump components are expressed by a wide range of bacteria colonisation or infection by which is associated with diseases or other deleterious conditions.

The medicaments of the invention provide a valuable alternative to anti-bacterial medicaments already known in the prior art. It is generally recognised that it is advantageous to have multiple anti-bacterial therapies effective against any particular deleterious bacterium, since bacterial populations may occasionally undergo spontaneous mutation that may render known treatments ineffective. The medicaments most commonly used in current treatments of bacterial infection or colonisation are vaccines or anti-bacterial agents such as antibiotics, however there are a number of disadvantages to such known medicaments and methods of treatment.

It is recognised that vaccines currently employed in the prevention and/or treatment of bacterial infection and/or colonisation may be rendered ineffective by mutations that cause the bacterial population to no longer express the epitope (or epitopes) responsible for generation of a protective immunisation response. Similarly antibiotic compounds that are presently used to control potentially harmful bacterial populations may also be rendered ineffective by mutations giving rise to antibiotic resistance. Indeed, the promiscuous use of antibiotics is widely considered to be one of the factors giving rise to the increased generation of so-called "superbugs", antibiotic-resistant bacterial populations that no longer respond to existing methods of treatment. The medicaments of the invention allow the generation of an expanded range of effective anti-bacterial medicaments, and thereby reduce the likelihood that mutation may render a deleterious bacterium incapable of treatment. The medicaments of the invention also provide a useful alternative to antibiotics, the extensive use of which in farming is increasingly viewed with concern by consumers seeking more "natural" products.

In a preferred embodiment a medicament in accordance with the first aspect of the invention comprises a bacteriophage capable of binding to a bacterial efflux component expressed by Gram negative bacteria.

Medicaments in accordance with this embodiment of the invention may comprise bacteriophage capable of binding to bacterial efflux pump components expressed by any

suitable genus or species of Gram-negative bacteria. For example, suitable bacteriophages may be capable of binding to bacterial efflux pump components expressed by genus or species of Gram-negative bacteria selected from the group comprising *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., *Haemophilus* spp., *Shigella* spp., *Yersinia* spp., *Vibrio cholerae*, and *Moraxella* spp. Preferably bacteriophages suitable for use in accordance with these embodiments of the invention are capable of binding to bacterial efflux pump components expressed by bacteria selected from the group comprising *Salmonella* spp. and *Campylobacter* spp.

In the light of the above the skilled person will appreciate that the medicaments of the invention may be used in the prevention and/or treatment of diseases caused by the activity of bacteria selected from the group consisting of *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., *Haemophilus* spp., *Shigella* spp., *Yersinia* spp., *Vibrio cholerae*, and *Moraxella* spp.

Preferably medicaments of the invention may be used in the prevention and/or treatment of diseases caused by the activity of bacteria selected from the group consisting of *Salmonella* spp. and *Campylobacter* spp.

The skilled person will thus appreciate that bacteriophages capable of binding to a bacterial efflux pump component may be used in the manufacture of medicaments for the prevention and/or treatment of Salmonellosis, Campylobacteriosis, diseases caused by *Escherichia coli*, Klebsiellosis, Pseudomoniasis, Enterobacteriosis, Serratiosis, Citrobacteriosis, Proteosis, Morganelliasis, Acinetobacteriosis, pathological colonisation by *Stenotrophomonas* spp., diseases (such as meningitis, epiglottitis, bacteremia or cellulitis) caused by *Haemophilus* spp., Shigellosis, Yersiniosis, cholera, and diseases (such as bronchitis, sinusitis, laryngitis and otitis media) caused by *Moraxella* spp.

Preferably bacteriophages capable of binding to a bacterial efflux pump component may be used in the manufacture of medicaments for the treatment of Salmonellosis or Campylobacteriosis.

The cytoplasm of Gram-negative bacteria is bounded by the plasma membrane, which is in turn enveloped by the outer membrane. Effective bacterial efflux pumps must allow the transport of substances from the interior of a bacterium to the extra-bacterial space. Accordingly, such pumps must enable the transport of substances across the two membranes surrounding bacteria. Suitable bacterial efflux pump components that may be bound by bacteriophages for use in accordance with invention may be selected with reference to the location of such components in intact bacteria.

The inventors have surprisingly found that efflux pump components that are associated with the bacterial inner membrane may be used as effective targets that may be bound by bacteriophage suitable for use in the medicaments of the invention. This finding is surprising since it may be expected that such efflux pump components, which are normally "hidden" from a host by the outer membrane of intact bacteria, would not normally constitute successful targets for therapeutically effective bacteriophages since the viruses would not have access to (and therefore not be able to react to) these components in live bacteria. In the light of the inventors' finding it may be preferred that bacteriophages for use in the medicaments of the invention may be capable of binding to efflux pump components associated with the inner membrane of intact bacteria. For example, in the case of the AcrAB-TolC efflux pump, bacteria capable of binding to AcrA or AcrB may advantageously be used.

As an alternative, bacteriophages suitable for use in the medicaments of the invention may be capable of binding to efflux pump components associated with the outer membrane of intact bacteria. In the case of the AcrAB-TolC efflux pump suitable bacteriophages may be capable of binding to the outer membrane channel TolC.

The inventors believe that bacteriophages that are capable of binding to the bacterial efflux pump component TolC, and thereby killing the bacteria to which they are bound, are particularly useful in the preparation of medicaments according to the invention.

The inventors have identified a number of specific bacteriophage strains that preferentially bind to and lyse salmonella expressing TolC. This activity is enhanced in respect of salmonella that over-express *tolC*, while no activity is observed in respect of salmonella in which *tolC* has been disrupted, and is not expressed. These results clearly indicate that these bacteriophages are capable of binding to the bacterial efflux pump component TolC. Based on this specificity of binding, and on their ability to lyse and kill the bacteria to which they bind, the use of these bacteriophages as medicaments are preferred embodiments of the invention. These bacteriophages may be used for the prevention and/or treatment of diseases caused by infection or colonisation by members of the Enterobacteriaceae family (which includes *E.coli*, *Salmonella* spp.; *Klebsiella* spp.; *Serratia* spp.; and *Haemophilus influenzae*), since these bacterial family members all express TolC. In preferred embodiments these bacteriophages may be used for the prevention and/or treatment of diseases caused by infection or colonisation by *Salmonella* spp., and in a particularly preferred embodiment one or more of these phage may be used for the prevention and/or treatment of diseases caused by infection and/or colonisation by *Salmonella Typhimurium*.

Among the bacteriophages that have been identified by the inventors as suitable for use in accordance with the present invention are a number of bacteriophages previously used in the "typing" of unknown bacteria.

Bacteriophage typing of bacteria such as *S. Typhimurium* has its beginnings fifty years ago, when Felix (1956) and Callow (1959) developed a system for phage typing strains of *Typhimurium* that has become established internationally as the method of choice for the epidemiological study of this most frequently isolated serotype. This system originally distinguished 34 phage types, and now has been progressively expanded. With a battery of 36 phages this system provides a very fine degree of discrimination for the 232 definitive types which are currently recognised.

Although many of the bacteriophages identified by the inventors were already well known to those skilled in the art (and were commercially available from suppliers such as the Health Protection Agency's Laboratory of Enteric Pathogens based at Colindale Avenue in London), and had been so for fifty years, it had not previously been realised that these bacteriophages were suitable for medicinal use. In particular it had not previously been recognised that these bacteriophages were capable of binding to efflux pump components such as TolC, and there had certainly been no previous indication that would have led the skilled person to believe that these bacteriophages were capable of use in the application described in the present specification (e.g. in the prevention and/or treatment of salmonellosis; campylobacteriosis; or other bacterial diseases as discussed elsewhere in the specification; or in the prevention or treatment of bacterial infections or colonisations in poultry). In the light of the above the disclosure of the present specification must be regarded as both new and very surprising.

The inventors have found that *S. Typhimurium* 'typing' bacteriophages 27, 29 and 35 are all able to bind to bacteria via the TolC efflux pump component, thereby causing the death of bacteria to which the phages bind, and are thus suitable for use in medicaments in accordance with the invention. Although the therapeutic utility of these bacteriophages was initially identified by the inventors on the basis of the phages' differential lysing activity for *S. Typhimurium* with different levels of TolC, the inventors believe that these phage may be of utility in the destruction of bacteria of other species or genera that also express TolC (or a very similar protein), such as *E. coli* or the like. Accordingly the inventors believe that bacteriophages selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35 may be used in the manufacture of medicaments for use in the prevention and/or treatment of diseases associated with infection or colonisation of any bacteria that express TolC. A suitable disease may preferably be one caused by colonisation or infection by *S. Typhimurium*, although these bacteriophages may be used to prevent and/or treat diseases caused by other forms of salmonella, or other bacteria.

The inventors have also found that *S. Enteritidis* typing bacteriophages 8 and 14 (also available from the Health Protection Agency's Laboratory of Enteric Pathogens) have preferential activity for salmonella with TolC, enhanced activity for salmonella over-expressing *tolC* and no activity for those in which *tolC* is disrupted, therefore suggesting that these bacteriophages are able to bind to bacteria via the efflux pump component TolC and thereby cause the death of bacteria so bound. Once again, although these bacteriophages were first identified in *S. Enteritidis*, the inventors believe that these bacteria may surprisingly be used in the prevention and or treatment of diseases associated with infection or colonisation by any bacteria that express TolC. The bacteriophages may even be used to prevent and/or treat diseases caused by bacteria expressing proteins very similar to TolC. A disease to be prevented and/or treated using *S. Enteritidis* typing bacteriophages 8 or 14 may preferably be one caused by colonisation or infection by *S. Enteritidis* or, surprisingly, one caused by *S. Typhimurium*, although these bacteriophages may also be used to prevent and/or treat diseases caused by other forms of salmonella, or by other bacteria.

In addition, the inventors have found that *S. Virchow* typing bacteriophage 12 (available from Health Protection Agency's Laboratory of Enteric Pathogens) has preferential activity for salmonella with TolC, enhanced activity for salmonella over-expressing *tolC* and no activity for those in which *tolC* is disrupted, therefore suggesting that this bacteriophage is able to bind to bacteria via TolC and to kill bacteria so bound. Thus *S. Virchow* bacteriophage may be used in the prevention and/or treatment of diseases caused by infection or colonisation by any bacteria that express TolC. The disease may preferably be one caused by colonisation or infection by *S. Virchow* or, surprisingly, one caused by *S. Typhimurium* or other salmonella or other bacteria.

In a further surprising finding, the inventors have found that *S. Hadar* typing bacteriophage 7 (available from Health Protection Agency's Laboratory of Enteric Pathogens) has preferential activity for salmonella with TolC, enhanced activity for salmonella over-expressing *tolC* and no activity for those in which *tolC* is disrupted, therefore suggesting that this bacteriophage is also capable of binding to bacteria through the efflux pump component TolC and thereby killing bacteria so bound. Once again, this

activity is not limited to *S. Hadar*, and is applicable to the prevention and/or treatment of diseases caused by the infection or colonisation of any bacteria expressing TolC. In a preferred embodiment the disease may be one that is caused by colonisation or infection by *S. Hadar* or, surprisingly, one caused by *S. Typhimurium* or other salmonella or other bacteria

It will be appreciated that non-limiting examples of bacteria that express TolC, and of the diseases caused by these bacteria, include:

Salmonella spp. which cause salmonellosis, a disease which may lead to nausea, vomiting, stomach cramps and diarrhoea in patients infected or colonised by such bacteria.

E. coli, which may cause diarrheal illness, hemolytic-uremic syndrome (HUS) urinary tract infections (UTIs) and neonatal sepsis and meningitis.

Other members of the Family Enterobacteriaceae including *Klebsiella* spp., *Serratia* spp., and *Enterobacter* spp, all of which can cause diarrheal illness, urinary tract infections (UTIs) and neonatal sepsis and meningitis.

Haemophilus influenzae, which may cause meningitis, epiglottitis, laryngitis, bacteraemia and cellulitis.

Gram negative bacteria expressing homologues of TolC include *P. aeruginosa* which can cause a variety of opportunistic infections in humans, including skin and soft tissue infections in patients with burns and pneumonia in individuals with cystic fibrosis and *Acinetobacter baumannii* which is a cause of human nosocomial infections, causing increasing problems in intensive care units particularly due to multi drug resistant strains. The inventors believe that diseases caused by infection or colonisation by these bacteria may be prevented and/or treated using the methods and medicaments of the invention.

Accordingly the inventors believe that any of the bacteriophages identified above (i.e. any of the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7) may be used in the manufacture of medicaments for the prevention and/or treatment of diseases outlined in the preceding paragraphs (i.e. diseases selected from the group consisting of salmonellosis; Klebsiellosis; Serratiosis; diseases caused by *E. Coli*; and diseases caused by *Haemophilus influenzae*).

The invention also provides a method of preventing and/or treating infection and/or colonisation by a bacterium expressing TolC, the method comprising administering to a subject in need of such prevention and/or treatment a therapeutically effective amount of a bacteriophage capable of binding the bacterial efflux pump component TolC. The bacteriophage may preferably be administered using a medicament of the invention.

Suitable bacteriophages may preferably be selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7. Bacteria the colonisation or infection of which may be prevented and/or treated using such methods include all members of the family Enterobacteriaceae, as considered elsewhere in the specification. The infection and/or colonisation by the bacterium expressing TolC may lead to a disease caused by a member of the Family Enterobacteriaceae including salmonellosis; and diseases caused by *E. coli*, *Serratia spp*, *Klebsiella spp*, *Enterobacter spp*; and diseases caused by *Haemophilus Influenzae*.

Medicaments in accordance with the invention may comprise two or more bacteriophages capable of binding to bacterial efflux pump components. Such medicaments may comprise two or more strains of bacteriophages capable of binding to the same bacterial efflux pump component. Alternatively, suitable medicaments may comprise two or more strains of bacteriophages capable of binding to different bacterial efflux pump components.

In the case where a medicament of the invention comprises two or more bacteriophage strains capable of binding to different bacterial efflux pump components the bacteriophage strains may recognise components of different bacterial efflux pumps, or different components of the same bacterial efflux pump. In the latter case suitable medicaments, for example, comprise one (or more) bacteriophage strain capable of binding to an efflux pump component associated with the inner membrane of intact bacteria and one (or more) bacteriophage strain capable of binding to an efflux pump component associate with the outer membrane of intact bacteria.

Many bacterial efflux pumps are known, amongst the best characterised of which is the AcrAB-TolC efflux pump (found in all bacteria in the Enterobacteriaceae family including *Escherichia coli*; *Salmonella* spp., *Klebsiella* spp., *Serratia* spp., and *Haemophilus influenzae*). Accordingly it is a preferred aspect of the invention that medicaments comprise bacteriophages capable of binding to components of the AcrAB-TolC bacterial efflux pump.

It will be appreciated that the use of medicaments in accordance with the invention comprising bacteriophages capable of binding to components of the AcrAB-TolC bacterial efflux pump is particularly beneficial since the AcrAB-TolC efflux pump components are expressed by many bacterial serovars, and hence medicaments in accordance with this embodiment of the invention are able to exert anti-bacterial activity across a range of different serovars.

The AcrAB-TolC efflux pump is a tripartite complex, its three component members being AcrA, AcrB and TolC. These three components associate to form a multi-component pump which functions to actively excrete harmful substances from the bacterial cytoplasm to the extra-bacterial space beyond the outer membrane.

AcrA is an approximately 42 kD membrane fusion lipoprotein also known as B0463, SipB, Mbl, Lir, NbsA, MtcA and the acridine efflux pump. AcrA has a periplasmic location in intact bacteria, and is anchored to the outer surface of the inner membrane by the lipid moiety of the lipoprotein. It is believed that AcrA and AcrB interact to form a

complex that is stable even in the absence of TolC. A small region at the C-terminus of AcrA has been shown to be necessary for the interaction of the protein with AcrB. The amino acid sequence of AcrA of *Salmonella typhimurium* is shown as Sequence ID No.1, and a nucleotide sequence encoding *acrA* as Sequence ID No.2. In a preferred embodiment a medicament of the invention may comprise a bacteriophage capable of binding to an AcrA protein such as the AcrA of *Salmonella typhimurium*.

AcrB is an approximately 113 kD RND-type permease also known as AcrE and B0462. AcrB is associated with the inner membrane of intact bacteria, and has twelve membrane-spanning α -helices. The presence of AcrB in the AcrAB-TolC efflux pump complex allows electrochemical-gradient energy to drive the active excretion of substances from the bacterial cytoplasm. The amino acid sequence of AcrB of *Salmonella typhimurium* is shown as Sequence ID No.3, and a nucleotide sequence encoding *acrB* as Sequence ID No.4. In a further preferred embodiment a medicament of the invention may comprise a bacteriophage capable of binding to an AcrB protein such as the AcrB of *Salmonella typhimurium*.

Both AcrA and AcrB are encoded by the single *acrAB* locus. In intact bacteria AcrAB (the complex of AcrA with AcrB) is able to extrude substances from the bacterial cytoplasm without their accumulation in the periplasmic space, indicating that AcrAB functions in conjunction with an outer membrane channel. Genetic and co-localisation studies have indicated that this channel is provided by TolC.

TolC is an approximately 54 kD bacterial porin also known as B3035, WeeA, Toc, Refl, MukA and MtcB. In vivo TolC is believed to form a functional trimer, with each monomer comprising a beta barrel of 18 membrane-spanning beta strands. The amino acid sequence of TolC of *Salmonella typhimurium* is shown as Sequence ID No.5, and a nucleotide sequence encoding *tolC* as Sequence ID No.6. In a still further preferred embodiment a vaccine of the invention may comprise a bacteriophage capable of binding to a TolC protein such as the TolC of *Salmonella typhimurium*. A particularly preferred bacteriophage capable of binding to TolC of *S. Typhimurium* may be selected from the

group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7.

In addition to bacteriophages capable of binding to components of the AcrAB-TolC bacterial efflux pump, the inventors have also found that bacteriophages capable of binding to components of the CmeABC efflux pump may also be used in the preparation of medicaments of the invention. CmeABC is a tripartite efflux pump normally expressed by *Campylobacter* spp. Preferred bacteriophage suitable for use in accordance with the present invention may therefore be those capable of binding to CmeA and/or CmeB and/or CmeC.

The inventors have further found that bacteriophages capable of binding to components of other bacterial efflux pumps systems are also suitable for use in accordance with the invention. By way of non-limiting example, such bacteriophages may include those able to bind to components of bacterial efflux pumps selected from the group comprising MexAB-OprM, MexCD-OprJ, MexXY-OprM, MexEF-OprN, MexJK-OprM (all *P. aeruginosa*), SmeDEF (*Stenotrophomonas maltophilia*), SdeAB, Sde XY (*Serratia marscecens*), and AdeABC (*Acenitobacter baumannii*).

The amino acid sequences (and corresponding DNA sequences) of a number of examples of components of bacterial efflux pumps that may be targeted by bacteriophages suitable for use in accordance with the invention are provided in Figure 1 that accompanies this application.

Preferably bacteriophages suitable for use in accordance with the invention are bacteriophages that have been engineered or selected to have an increased affinity for bacterial efflux pump components. For the purposes of the present invention engineering may be taken to encompass any artificial means by which bacteriophage having increased affinity for bacterial efflux pump components may be produced. Examples of suitable means by which such bacteriophages may be produced will be readily apparent to those of skill in the art.

Suitable bacteriophages that may be used in the medicaments and methods of the invention may be produced by the mutation of existing bacteriophage populations. Such mutations may be produced using chemical, radiological or other methods well known to those skilled in the art. Mutations may also be made by homologous recombination methods well known to those skilled in the art.

Naturally occurring, mutant or engineered bacteriophages may be screened for the ability to bind bacterial efflux pump components (for example using the methods described below), and those with useful characteristics may then be selected for use in the methods and medicaments of the invention. By way of example, useful characteristics may include an increased affinity for bacterial efflux pump components, an increased ability to kill bound bacteria, or the ability to infect previously resistant bacterial strains.

Bacteriophages having increased affinity for bacterial efflux pump components may be selected by means well known to those skilled in the art. For example, it is known that the target specificity of a bacteriophage may be altered by the choice of substrate on which it is grown. That is, two genetically identical bacteriophages may exhibit different target specificity when they have been grown on different substrates. Thus bacteriophages having required target specificity may be selected from those grown on substrates containing high levels of suitable bacterial efflux pump components.

Bacteriophages suitable for use in accordance with the invention are preferably those that are capable of binding to an efflux pump component expressed by bacteria associated with a deleterious condition or disease. Such conditions or diseases may arise as a result of either bacterial infection or colonisation of a host. Preferably, a bacteriophage suitable for use in accordance with the present invention is one capable of binding to an efflux pump component expressed by bacteria associated with deleterious conditions of humans. In another preferred embodiment a bacteriophage suitable for use in accordance with the present invention is one capable of binding to an efflux pump component expressed by bacteria associated with deleterious conditions of poultry.

It will be appreciated that medicaments in accordance with the invention should be administered so as to provide an effective anti-bacterial dose. For the purposes of the present invention an effective anti-bacterial dose should be considered to be a dose of a medicament in accordance with the present invention sufficient to prevent or treat a disease or condition caused by bacterial infection and/or colonisation. Preferably an effective anti-bacterial dose of a medicament in accordance with the invention may be sufficient to render a subject to whom the medicament is administered substantially free from bacterial infection and/or colonisation associated with the condition or disease to be treated. Effective anti-bacterial activity may arise as a result of the ability of bacteriophage to infect bacterial species associated with deleterious conditions or diseases, and to have a detrimental effect on the infected bacterial cells. This will normally give rise to the death of some or all of the infected cells. Preferably the bacteriophages will have activity against target bacterial species responsible for the deleterious condition or disease, but will have no activity or lower activity against other non-damaging bacterial species.

The ability of bacteriophage-containing medicaments of the invention to exert anti-bacterial activity may be readily investigated using methods well known to those skilled in the art. For example, a test sample comprising bacteria against which it is desired to exert anti-bacterial activity may be cultured on a solid growth medium, such as on an agar plate. The sample is preferably cultured on said medium for a sufficient time and under suitable conditions for target bacteria present in the sample to multiply on the surface of the plate. By contacting the surface of the plate with a medicament in accordance with the invention, it can be determined whether any of the bacteriophages thus added are capable of infecting and killing the bacteria. The bacteriophage-infected medium may be maintained under suitable conditions for bacteriophage infection and replication, such that the bacteriophages have an opportunity to infect target bacteria present on the plate. This will lead to the development of clear patches (plaques) where bacterial death has occurred, and will indicate that the test sample contained the target bacterial species.

In an alternative assay, the test sample of bacteria may be maintained in a liquid medium. Again, it may be cultured under conditions suitable for bacterial growth. Following the

addition of a medicament in accordance with the invention, the medium may be maintained for a further period to allow the bacteriophages to infect any target bacteria present. This will lead to a loss of turbidity in the medium when bacterial death occurs, and this will indicate that bacteriophages present in the medicament of the invention are able to exert an anti-bacterial activity against bacteria present in the test sample.

It will be recognised that one of the advantages of the medicaments of the present invention lies in the fact that they employ replicating biological agents which have the ability to generate an effective therapeutic dose as and when needed, even from a very low input dose.

It is recognised that administration of an effective anti-bacterial dose may be achieved by way of a single administration (i.e. administration of a single dose of a medicament, said dose constituting an effective anti-bacterial dose), or by way of multiple administration (i.e. administration of two or more doses of a medicament, said two or more doses combining to constitute an effective anti-bacterial dose).

Medicaments in accordance with the present invention may preferably be provided in the form of dosage units. For example, in the case where a medicament is to be administered such that a single administration is sufficient to provide an effective anti-bacterial dose, such a single administration may constitute a suitable dosage unit. Alternatively, when multiple administrations of a medicament are to be required in order to provide an effective anti-bacterial dose (for example a first "primer" and second "booster" administration) a suitable dosage unit may comprise a sufficient quantity of the medicament to provide the necessary administrations.

The amount of a suitable bacteriophage to be contained in a dose of a vaccine of the invention in order to provide an effective immunising dose may be determined with reference to a number of factors. Suitable factors may include the virulence of the bacteriophages of the medicament; the efficiency with which the bacteriophages are able to kill their bacterial targets; the affinity of the bacteriophages for the bacterial efflux pump components; the route of administration by which the vaccine is administered; the

relative size of the subject receiving the medicament; and the extent of bacterial infection or colonisation from which the subject may suffer, either before or after administration of the medicament.

Preferably bacteriophages of medical interest (and hence suitable for use in the medicaments and methods of treatment of the invention) are those that on binding to a bacterial efflux pump component are able to kill bacteria expressing said efflux pump component.

Medicaments in accordance with the present invention may be administered to a subject by any suitable route of administration known in the prior art.

Medicaments in accordance with the invention may preferably comprise a bacteriophage capable of binding to a bacterial efflux pump component, in combination with a pharmaceutical carrier or diluent carrier. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, transdermal, ocular or aural administration, e.g. a liquid formulation for administration as eye or ear drops. Such a bacteriophage preparation may be used directly, stored frozen in aqueous or other solution with an appropriate cryoprotectant (e.g. 10% sucrose), freeze dried and rehydrated prior to use, or rendered stable in some other formulation including (but not limited to) tablet, emulsion, ointment, or impregnated wound dressing or other item.

Bacteriophage preparations and compositions on the invention may be administered to a patient topically, systemically, orally, or by other means suitable for delivering an effective anti-bacterial dose to the site of bacterial colonisation or infection to be treated. The administration of medicaments in accordance with the invention will be in such a way that the bacteriophage can be incorporated into bacteria at the site of the infection. The routes of administration and dosages described below are provided only by way of example. In practice, a skilled practitioner will be able to determine the optimum route of administration and dosage for any particular patient and condition.

In the case that medicaments of the invention are administered orally to patients it may be preferred that the patients pre-treated with antacids and/or gelatin to prevent destruction of the bacteriophages by gastric acid.

It may generally be preferred that medicaments in accordance with the present invention be administered orally. Orally administered medicaments are generally safer for patients or subjects receiving the medicament, and the administration of such medicaments requires little by way of specialised training. Both physicians and patients may typically favour the use of oral medicaments since such medicaments are often cheaper and easier to deliver than injectable medicaments. In particular, the administration of oral medicaments does not generally require the use of sterilising equipment, and further involves no pain on administration of such medicaments to a patient or subject.

The use of orally administered medicaments is particularly preferred in the case that the medicaments are for the prevention and/or treatment of bacterial colonisation and/or infection occurring in the digestive tract.

When medicaments in accordance with the present invention are to be administered by the oral route they may make use of any suitable formulation known to those skilled in the art. Medicaments for oral administration may preferably be formulated such that the bacteriophages present in the medicaments are protected from the harsh conditions of the digestive tract, which may otherwise cause the bacteriophages to be degraded. Suitable methods by which such protection may be conferred are well known to those skilled in the art and include encapsulation, microencapsulation, and/or the use of enteric coatings.

Oral administration of medicaments of the invention may be achieved by inoculation (such as by oral gavage) or by application of the medicaments in drinking water. Application in drinking water may represent a preferred route of oral administration since the medicaments may be administered to a relatively large number of subjects without extensive intervention by a person administering the medicaments.

As an alternative to their oral administration, suitably formulated medicaments may be administered to a subject by means of injection. In particular, medicaments in accordance with the present invention may be administered by intramuscular injection, intradermal injection subcutaneous injection, or intravenous injection. Formulations for use in the preparation of injectable medicaments are well known to those of skill in the art.

Medicaments in accordance with the present invention may also be administered by inhalation, for example via intranasal spray. It is well known to provide medicaments by nasal inhalation and such administration may be preferred since it lacks many of the undesirable effects associated with administration by injection (such as injection pain and the requirement for sterilising equipment). Suitable nasal spray formulations which may be used in the preparation of medicaments in accordance with the present invention will be known to those skilled in the art.

It has recently been shown that effective doses of vaccines may be administered to poultry through the use of whole body sprays. Surprisingly aerosol immunisation in this manner has been found to be suitable for the generation of a systemic immune response, not just a response associated with the respiratory tract. Accordingly, the use of such whole body sprays represents a preferred route of administration, particularly in the case of administration of medicaments in accordance with the present invention to poultry.

The use of routes of administration other than oral administration may be preferred in the case where it is desired to administer the vaccines of the invention to young poultry. Newly hatched chicks are known not to eat or drink for up to several days after their hatching, and so the use of injection, inhalation, or whole body spray may be particularly preferred in the case where it is desired to provide the medicament to such relatively young poultry. Other suitable methods for administration of medicaments of the present invention to poultry include administration by means of eye drop or injection.

Generally when medicaments in accordance with the invention are used to prevent bacterial colonisation or infection the medicament should be administered as soon as the risk of bacterial colonisation or infection has been identified.

The amount of bacteriophage administered will depend upon the size, location and nature of the area to be treated and the route of administration used. As a successful treatment will lead to multiplication of the bacteriophages and killing of infected bacteria, some treatments, for example those requiring topical infection, may only require the administration of a relatively low dose of bacteriophages in order to provide an effective anti-bacterial dose. The number of bacteriophages required to provide an effective anti-bacterial dose may be defined with reference to the ability of the bacteriophages to form cleared zones or "plaques" on bacterial culture plates. Such units are defined as "plaque forming units" or "pfu". In some cases an effective anti-bacterial dose of a medicament in accordance with the invention may comprise a few hundred infectious units (pfu) or less. A suitable dose may be 10^2 to 10^8 pfu, and preferably 10^4 , to 10^6 pfu. In other cases, for example in the case of systemic or widespread colonisation or infection, the dose may need to be higher to ensure that the bacteriophages reach all sites at which bacteria are present. In such a case a suitable dose may be in the range of from 10^4 to 10^{10} pfu, preferably from 10^5 to 10^8 pfu. When injected, typically 10 μ l to 50 ml of bacteriophages in a pharmaceutically acceptable suitable carrier or diluent is administered. For topical administration the volume may be higher, for example 100 μ l to 50 ml of the medicament, depending on the size, location and nature of the area to be treated.

It is generally preferred that when producing medicaments comprising bacteriophage a high phage titer should be obtained. As described above, administration of therapeutically effective bacteriophage compositions can be performed by many routes, including but not limited to oral, aerosol, nasal spray, intravenous, intramuscular, intraperitoneal, intraperitoneal, intrathecal, vaginal, rectal and topical administration. It may generally be preferred that, irrespective of the route of administration to be used, the titer of the phage should be at the range of 10^{10} to 10^{13} pfu/Kg body weight/day, preferably about 10^{11} to 10^{12} pfu/Kg body weight/day.

The ability of bacteriophages capable of binding to bacterial efflux pump components to exert suitable anti-bacterial activity may be investigated by monitoring the effects of the

bacteriophages on bacterial cells. Typically this may involve determining bacterial death by observing the formation of cleared zones in bacteria grown on solid substrates ("plaques") or a loss of turbidity in liquid culture.

Frequency of administration will depend upon on, among other considerations, the biological half-life of the agent used. Typically a medicament of the invention should be administered such that the concentration of the bacteriophage at a tissue at risk of bacterial colonisation or infection is maintained at a level sufficient to achieve an effective anti-bacterial dose.

Medicaments of the invention, may be administered by any suitable route capable of achieving the desired effect of preventing or reducing bacterial colonisation or infection, but it is preferred that the medicaments be administered orally, or by injection or inhalation.

Medicaments of the invention may be used to prevent or reduce bacterial infection or colonisation as a monotherapy (e.g. through use of the medicaments of the invention alone). Alternatively the methods or medicaments of the invention may be used in combination with other compounds or treatments to prevent or reduce bacterial infection or colonisation. Suitable treatments that may be used as parts of such combination therapies will be well known to those skilled in the art, and may include the use of applicable antibiotic and antimicrobial agents.

In a further aspect of the invention there is provided a method of preventing, treating or reducing a disease associated with bacterial colonisation or infection, the method comprising administering to a patient in need of such treatment or reduction an effective amount of a bacteriophage capable of binding to a bacterial efflux pump component expressed by the colonising or infecting bacteria. A patient in accordance with this aspect of the invention may be any human or non-human animal in need of treatment or reduction of disease. Preferred bacteriophages may be selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7.

An “effective amount” of a bacteriophage suitable for use in accordance with this aspect of the invention may be an amount sufficient to prevent, treat or reduce a disease associated with bacterial colonisation or infection. An effective amount may preferably be sufficient to render the patient so treated substantially free from bacteria associated with the disease or condition.

It will be appreciated that medicaments in accordance with the present invention may be used in the production of animal-based foodstuffs, since the medicaments are able to prevent and/or treat bacterial colonisation and/or infection that may otherwise give rise to the harmful presence of bacteria in foodstuffs.

Accordingly, in a further aspect, the invention provides a method of preparing meat for human consumption, the method comprising:

- i) administering to an animal intended for meat production an amount of a bacteriophage capable of binding to a bacterial efflux pump component sufficient to render the animal substantially free of a Gram-negative bacterium harmful to humans;
- ii) growing the animal to a size suitable for harvesting for meat production; and
- iii) harvesting the animal for meat production.

In a still further aspect, the invention provides a method of preparing food for human consumption, the method comprising:

- i) administering to an animal intended for food production an amount of a bacteriophage capable of binding to a bacterial efflux pump component sufficient to render the animal substantially free of a Gram-negative bacterium harmful to humans;
- ii) growing the animal to a size suitable for harvesting food from the animal; and
- iii) harvesting food from the animal for human consumption.

The bacteriophages to be administered in accordance with the preceding aspects of the invention may preferably be selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7.

Animal-based foodstuffs in the context of the present invention may be taken to encompass both the meat of animals and other foodstuffs produced by animals, such as eggs, milk or the like. The term animal-based foodstuffs should also be taken to encompass products derived from foods produce by animals, such as dairy produce derived from milk. The methods of the invention are suitable for use with all farmed animals, including poultry, cattle, pigs, sheep and fish.

It is known that administration of medicaments to food animals by injection may be associated with the development of lesions in the carcasses of animals so injected. Therefore it may be preferred that bacteriophage-containing medicaments administered in accordance with the methods of the preceding aspects of the invention be administered by a needle-free route of administration.

In a still further aspect of the invention there is provided a method of rendering a subject substantially free of a deleterious bacterium, the method comprising administering to the subject an effective amount of a medicament in accordance with the present invention. It should be recognised that in the context of this aspect of the invention a deleterious bacterium is a bacterium capable of having deleterious effects on humans, particularly through infection or colonisation of human hosts. The deleterious bacterium need not have a deleterious effect on the subject to whom the medicament is administered.

Bacteriophages selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7 may be used in the prevention and/or treatment of infection and/or colonisation by bacteria expressing TolC. Such bacteria may be members of the Enterobacteriaceae family, as considered elsewhere in the specification. It will be appreciated that, although the infection and/or colonisation by the bacteria expressing TolC may cause a disease, it may also be desirable to prevent and/or treat infections and/or colonisations that are not harmful to the host, but that may otherwise prove harmful to a human coming into contact with an animal host to such an infection and/or colonisation. For example, the bacteriophages may be used to prevent and/or treat an

infection and/or colonisation of an animal that will provide human food. Such food may be a direct product of the animal (such as meat, milk or eggs) or an indirect product of the animal (such as cheese or other dairy produce). The prevention and/or treatment may preferably be effected by administration of a medicament in accordance with the invention.

The invention also provides, in a further aspect, a method of identifying a bacteriophage of medical interest, the method comprising:

- i) exposing a population of bacteriophages to a bacterial efflux pump component expressed by a bacterium associated with a disease or condition characterised by bacterial colonisation and/or infection;
- ii) assaying for binding of bacteriophages of the bacteriophage population that bind to the bacterial efflux pump component; and
- iii) recovering bacteriophages that bind to the bacterial efflux pump component

wherein a bacteriophage recovered from iii) is considered to be of medical interest.

Preferably a bacteriophage identified in accordance with this aspect of the invention may be one capable of killing bacteria to which it binds by means of the bacterial efflux pump component(s), and accordingly the method may preferably include a further step of assaying for anti-bacterial activity of the bacteriophages so identified.

The invention will now be further described with reference to the accompanying Experimental Results section, and accompanying Figure 1, which sets out amino acid and DNA sequences of a number of bacterial efflux pump components that may be bound by preferred bacteriophages suitable for use in accordance with the invention.

EXPERIMENTAL RESULTS

STUDY A

Materials and Methods

Bacterial Strains.

For all *Campylobacter* assay experiments, *C. jejuni* NCTC 11168 (P270) and NCTC 11168-H (hyper motile) (P1712) were used as they are amenable to genetic manipulation and have been used to construct all of our mutants (Pumbwe, 2004). P1048 was used as it over-expresses CmeB and CmeC, bacterial efflux pump components that may be targeted by phage suitable for use in the methods and medicaments of the invention. NCTC 11168-H colonises poultry (Loc Carillo *et al.*, 2005), and so phage able to target this bacterium may be particularly preferred for use in the prevention and/or treatment of colonisation in poultry.

For all *Salmonella* assay experiments, the widely used strain of *S. enterica* serovar Typhimurium SL1344 was used (Wray, 1978). The *tolC* disrupted mutant (L108) (Buckley *et al.*, 2006) and TolC over expresser were constructed from SL1344.

Bacteriophage.

All the *Campylobacter* bacteriophages used in this study were obtained from Professor Ian Connerton, University of Nottingham. The phage are further described in Connerton *et al.*, 2004, and El-Shibiny *et al.*, 2005, both of which are incorporated herein by reference (particularly in respect of these documents identification of the phage described in the present specification)

All *Salmonella* bacteriophages used in this study were obtained from the Health Protection Agency (Colindale Avenue, London).

Construction of a strain of *S. enterica* serovar Typhimurium over-expressing TolC.

PCR was performed to amplify the entire *tolC* gene from initiation codon. The *tolC* gene was then ligated into pBAD and transformed into *E. coli* TOP10 competent cells and grown on LB agar containing 100µg/mL ampicillin. After overnight incubation at 37°C plasmid preparation from *E. coli* TOP10 was performed. PCR checks with external pBAD specific primers and internal *tolC* primers was performed and amplicons were sent for DNA sequencing to verify correct gene insert. Electroporation was used to introduce the pBAD-TolC construct into *S. Typhimurium* SL1344, recovery was performed on LB agar containing 100µg/mL ampicillin and verification on XLD of recovery of salmonella. The pBAD-TolC construct in SL1344 was then grown at various concentrations of arabinose to establish the concentration of arabinose to give optimum *tolC* expression. RNA expression was observed by performing RT-PCR and protein expression by performing SDS-PAGE.

Bacteriophage assay (Overlay method).

NZCYM (Sigma UK) agar plates at 8% agar concentration and 4% agar concentration (top agar) were used. To 10ml Brain Heart Infusion (BHI) broth (Oxoid) 100µL of 1M magnesium sulphate and 100µL 100mM calcium chloride were added. A suspension with a turbidity of 3 on McFarland's scale was prepared from an overnight culture of *Campylobacter* or a *Salmonella* culture at mid-logarithmic phase was made. 300µL of this suspension was added to the molten top agar and poured over the agar plates. When set and completely dry 10 – 20 µL of bacteriophage suspension at 10^9 pfu/mL were applied to the agar plates. Agar plates were incubated at 37°C in a micro-aerophilic environment (90% Nitrogen, 5% Carbon Dioxide and 5% Oxygen) for 24 hours. Following incubation the agar plates were observed for semi-confluent or confluent lysis.

Screening of *Salmonella* typing phages.

Salmonella enterica serovar Typhimurium strains SL1344 and L108 (*tolC::aph*) were sent to the Veterinary Laboratory Agency (VLA) to be assayed with a variety of *S. enterica* bacteriophages. The bacteriophages used were those routinely used by the VLA to type *Salmonella*.

Results

Campylobacter

Sixty out of the eighty campylobacter bacteriophages assayed were active against the three strains of Campylobacter examined: 11168, 11168-H and P1048 (over-expresses both CmeB and CmeC), showing confluent lysis at a titre of 10^9 pfu/mL. All three strains were resistant to the remaining twenty bacteriophages.

Salmonella

Of the thirty *S. Typhimurium* bacteriophages assayed three bacteriophages were not active against L108 but active against SL1344 with semi confluent lysis.

S. Typhimurium bacteriophages 27, 29 and 35 were further tested on three separate occasions at Birmingham confirming these data. Of the sixteen *Salmonella* Enteritidis bacteriophages assayed, two bacteriophages were not active against L108 but were active against SL1344 with semi confluent lysis; these were *S. Enteritidis* bacteriophages 8 and 14. Of the fifteen *Salmonella* Virchow bacteriophages assayed only one bacteriophage not active for L108 and active against SL1344 with semi confluent lysis: *S. Virchow* bacteriophage 12. Of the nine *Salmonella* Hadar bacteriophages assayed only one bacteriophage was not active for L108 and active against SL1344 with semi confluent lysis: *S. Hadar* bacteriophage 7.

Conclusions

1. All campylobacter bacteriophages studied were active against *C. jejuni* 11168.
2. Three phages used to type *S. Typhimurium* had activity suggestive of a phage that targets TolC (elucidated further in Study B below). Four other phages used to type other serovars of salmonella also had activity suggestive of

targeting TolC. All seven of these phages warrant further characterisation, and constitute phages that may be used in the methods and medicaments of the invention.

STUDY B

Phage titres of the three *S.Typhimurium* phages showing differential activity for strains with and without TolC.

The phage titres of three bacterial strains of *S. Typhimurium* were investigated. The experimental bacterial strains employed were as follows:

- i) wildtype SL1344;
- ii) L729, a variant form of SL1344 over-expressing TolC (pBAD*tolC*); and
- iii) L108, a mutant of SL1344 in which *tolC* is disrupted meaning that the protein TolC is not expressed.

tolC was cloned into pBAD to give strain L729. Arabinose induces expression of the cloned gene as an arabinose sensitive promoter is present in pBAD. At 0.00002µg/ml arabinose, *tolC* expression was five-fold higher than in SL1344.

Titres yielded by these bacterial strains were compared, and the results were as shown in the Table below.

Strain	Construct	Phage and titre (pfu/ml)		
		27	29	35
SL1344		10 ⁶	10 ⁵	10 ⁶
L729	SL1344 pBAD <i>tolC</i>	10 ⁵	10 ²	10 ⁴
L108	<i>tolC::aph</i>	0	0	0

These results show that phages 27, 29 and 35 had no activity when TolC was absent from bacteria. In addition, L729 was more susceptible than SL1344 to the three phages, and especially so to phage 29. These data further support the inventors finding that the three phages identified above interact with TolC and may be used in medicaments capable of preventing and/or treating disorders caused by infection or colonisation by bacteria expressing TolC.

SELECTED REFERENCES

Callow, B.R. "A new phage-typing scheme for *Salmonella typhi*-murium." J Hyg (Lond). 1959 Sep;57:346-59.

Felix, A. "Phage typing of *Salmonella typhimurium*: its place in epidemiological and epizootiological investigations." J Gen Microbiol. 1956 Feb;14(1):208-22.

Connerton PL, Loc Carrillo CM, Swift C, Dillon E, Scott A, Rees CE, Dodd CE, Frost J, Connerton IF. "Longitudinal study of *Campylobacter jejuni* bacteriophages and their hosts from broiler chickens." Appl Environ Microbiol. 2004 Jul;70(7):3877-83.

El-Shibiny A, Connerton PL, Connerton IF. "Enumeration and diversity of campylobacters and bacteriophages isolated during the rearing cycles of free-range and organic chickens." Appl Environ Microbiol. 2005 Mar;71(3):1259-66.

CLAIMS.

1. A bacteriophage capable of binding to a bacterial efflux pump component for use as a medicament.
2. A bacteriophage according to claim 1, wherein the bacteriophage is capable of binding to an efflux pump component expressed by Gram-negative bacteria.
3. A bacteriophage according to claim 2, wherein the bacteriophage is capable of binding to bacterial efflux pump components expressed by genus or species of Gram-negative bacteria selected from the group comprising *Salmonella* spp., *Campylobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Acinetobacter* spp., *Stenotrophomonas* spp., *Haemophilus* spp., *Shigella* spp., *Yersinia* spp., *Vibrio cholerae*, *Pseudomonas aeruginosa*, and *Moraxella* spp.
4. A bacteriophage according to any preceding claim, wherein the bacteriophage is capable of binding to efflux pump components associated with the inner membrane of intact bacteria
5. A bacteriophage according to any of claims 1 to 3, wherein the bacteriophage is capable of binding to efflux pump components associated with the outer membrane of intact bacteria
6. A bacteriophage according to any preceding claim, wherein the bacteriophage is capable of binding to components of the AcrAB-TolC bacterial efflux pump.
7. A bacteriophage according to claim 6, wherein the bacteriophage is capable of binding to AcrA.
8. A bacteriophage according to claim 6, wherein the bacteriophage is capable of binding to AcrB.

9. A bacteriophage according to claim 6, wherein the bacteriophage is capable of binding to TolC.
10. A bacteriophage according to selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7
11. A bacteriophage according to any of claims 1 to 5, wherein the bacteriophage is capable of binding to components of the CmeABC bacterial efflux pump.
12. A bacteriophage according to claim 11, wherein the bacteriophage is capable of binding to CmeA.
13. A bacteriophage according to claim 11, wherein the bacteriophage is capable of binding to CmeB.
14. A bacteriophage according to claim 11, wherein the bacteriophage is capable of binding to CmeC
15. A bacteriophage according to any of claims 1 to 5, wherein the bacteriophage is capable of binding to components of bacterial efflux pumps selected from the group comprising MexAB-OprM, MexCD-OprJ, MexXY-OprM, MexEF-OprN, MexJK-OprM, SmeDEF, SdeAB, Sde XY, and AdeABC.
16. The use of a bacteriophage according to any preceding claim for use in the manufacture of a medicament for the prevention and/or treatment of a disease caused by infection and/or colonisation by a Gram-negative bacteria.
17. The use according to claim 16, for the manufacture of a medicament for the prevention and/or treatment of a disease selected from the group consisting of salmonellosis; and diseases caused by members of the Family Enterobacteriaceae

including *E. coli*, *Serratia spp*, *Klebsiella spp*, *Enterobacter spp*; and diseases caused by *Haemophilus Influenzae*

18. A method of preparing meat for human consumption, the method comprising:

- i) administering to an animal intended for meat production an amount of a bacteriophage capable of binding to a bacterial efflux pump component sufficient to render the animal substantially free of a Gram-negative bacterium harmful to humans;
- ii) growing the animal to a size suitable for harvesting for meat production; and
- iii) harvesting the animal for meat production.

19. A method of preparing food for human consumption, the method comprising:

- i) administering to an animal intended for food production an amount of a bacteriophage capable of binding to a bacterial efflux pump component sufficient to render the animal substantially free of a Gram-negative bacterium harmful to humans;
- ii) growing the animal to a size suitable for harvesting food from the animal; and
- iii) harvesting food from the animal for human consumption.

20. A method according to claim 18 or claim 19, wherein the animal is a cow.

21. A method according to claim 18 or claim 19, wherein the animal is a poultry animal.

22. A method according to any one of claims 18 to 21, wherein the bacteriophage is selected from the group consisting of *S. Typhimurium* bacteriophages 27, 29 and 35; *S. Enteritidis* bacteriophages 8 and 14; *S. Virchow* bacteriophage 12; and *S. Hadar* bacteriophage 7.

23. A method of identifying a bacteriophage of medical interest, the method comprising:

- i) exposing a population of bacteriophages to a bacterial efflux pump component expressed by a bacterium associated with a disease or condition characterised by bacterial colonisation and/or infection;

- ii) assaying for binding of bacteriophages of the bacteriophage population that bind to the bacterial efflux pump component; and
 - iii) recovering bacteriophages that bind to the bacterial efflux pump component
- wherein a bacteriophage recovered from iii) is considered to be of medical interest.

MNKNRGLTPLAVVLM LSGSLALTGCDDKQDQGGQOMPEVGVVTLKTEPLQITTELPGR
 VAYRIA EVRPQVSGIILKRN FVEGSDIEAGVSLYQIDPATYQATYDSAKGDLAKAQA
 IAE LTVKRYQKLLGTQYISKQEYDQALADAQQATAAVVAAKAAVETARINLAYTKVTSPI
 SGRIGKSSVTEGALVQNGQASALATVQQLDPIYVDVTQSSNDFLRLKQELANGSLKQENG
 KAKVDLVTS DGIKFPQSGTLEFSDVTVDQTTGSITLRAIFPNPDHTLLPGMFVRARLQEG
 TKPTALLVPQQGVTRTPRGDATVLVVGADNKVETRQIVASQAIGDKWLVT DGLKAGDRV
 VSGLQKVRPGAQVKVQEITADNKQQAASGDQPAQPRS

Sequence ID No. 1 – amino acid sequence of AcrA of Salmonella Typhimurium LT2

ATGAACAAAAACAGAGGGTTAACGCCTCTGGCGGTCGTTCTGATGCTCTCAGGCAGCTTA
 GCGCTAACAGGATGTGACGACAAACAGGACCAGCAAGGCGGCCAGCAGATGCCAGAAGTT
 GGGGTTGTCTACACTAAAAACGGAACCACTGCAAATCACAACTGAACCTCCGGGTCGTACC
 GTTGCTTACCGTATCGCCGAAGTTCCGCCCGCAGGTAAGCGGCATTATCCTGAAGCGTAAT
 TTCGTTGAGGGAAGTGATATCGAAGCGGGAGTCTCTCTCTATCAGATTGATCCTGCGACC
 TACCAGGCGACTTACGACAGCGCTAAGGGCGATCTGGCAAAAGCGCAGGCCGCCGCGAAT
 ATCGCTGAAC TGACGGTGAAGCGTTATCAAAAGCTGCTGGGTACGCAGTACATCAGTAAG
 CAGGAATACGATCAGGCGCTGGCTGACGCACAACAAGCGACTGCCGCTGTTGTGCGAGCA
 AAAGCCGCCGTTGAAACCGCACGTATCAACCTGGCGTATACCAAAGTCACCTCGCCGATT
 AGCGGTCGTATTGGTAAGTCATCCGTAACGGAAGGCGCACTGGTACAGAACGGTCAGGCG
 TCGGCGCTGGCGACAGTGCAGCAGCTGGACCCCTATTTATGTCGATGTGACCCAGTCCAGC
 AATGACTTCCTGCGCCTGAAGCAGGAGCTGGCAAATGGTTCGCTGAAACAGGAAAACGGC
 AAAGCGAAGGTCGACCTGGTGACCAGCGACGGTATCAAATCCCGCAGTCCGGTACGCTT
 GAGTTCTCCGACGTGACCGTTGACCAAACACCGGGTCTATTACTTTGCGCGCCATCTTC
 CCTAACCCGGATCACACCTTATTGCCAGGAATGTTTCGTTTCGCGCACGTCTGCAGGAAGGG
 ACAAACCGACGGCATTTACTGGTTCCACAACAGGGCGTTACCCGTA CTCCACGCGGCGAT
 GCCACGGTGCTGGTGGTTGGCGCTGATAACAAAGTGGAACCCGCCAAATCGTCGCAAGC
 CAGGCGATCGGCGATAAGTGCTGGTGACTGACGGATTGAAAGCGGGCGACCGCGTAGTC
 GTCAGCGGGCTGCAAAAAGTACGTCCTGGCGCACAGGTTAAAGTACAGGAAATTACCGCG
 GATAACAAACAGCAAGCCGCAAGCGGTGATCAACCTGCTCAGCCCAGGTCTTAA

Sequence ID No. 2 – DNA encoding AcrA of Salmonella Typhimurium LT2

MNKNRGFTPLAVVLM LSGSLALTGCDDKQAQGGQOMPAVGVVTVKTEPLQITTELPGR
 SAYRIA EVRPQVSGIILKRN FKEGSDIEAGVSLYQIDPATYQATYDSAKGDLAKAQA
 IAQLTVNRYQKLLGTQYISKQEYDQALADAQQANA AVTAAKAAVETARINLAYTKVTSPI
 SGRIGKSNVTEGALVQNGQATALATVQQLDPIYVDVTQSSNDFLRLKQELANGTLKQENG
 KAKVSLITSDGIKFPQDGTLEFSDVTVDQTTGSITLRAIFPNPDHTLLPGMFVRARLEEG
 LNPNAILLVPQQGVTRTPRGDATVLVVGADDKVETRPIVASQAIGDKWLVT EGLKAGDRV

Sequence ID No. 3 – amino acid sequence of AcrA of E. coli MG1655

Figure 1

ATGAACAAAAACAGAGGGTTTACGCCTCTGGCGGTCTGATGCTCTCAGGCAGCTTA
GCCCTAACAGGATGTGACGACAAACAGGCCCAACAAGGTGGCCAGCAGATGCCCGCCGTT
GGCGTAGTAACAGTCAAACTGAACCTCTGCAGATCACAACCGAGCTTCCGGGTGCGACC
AGTGCCTACCGGATCGCAGAAGTTCGTCTCTCAAGTTAGCGGGATATCCTGAAGCGTAAT
TTCAAAGAAGGTAGCGACATCGAAGCAGGTGTCTCTCTCTATCAGATTGATCCTGCGACC
TATCAGGCGACATACGACAGTGCAGAAAGGTGATCTGGCGAAAGCCCAGGCTGCAGCCAAT
ATCGCGCAATTGACGGTGAATCGTTATCAGAACTGCTCGGTACTCAGTACATCAGTAAG
CAAGAGTACGATCAGGCTCTGGCTGATGCGCAACAGGCGAATGCTGCGGTAAGTGCAGGCG
AAAGCTGCCGTTGAAACTGCGCGGATCAATCTGGCTTACACCAAAGTCACCTCTCCGATT
AGCGGTGCGCATTTGGTAAGTCGAACGTGACGGAAGGCGCATTGGTACAGAACGGTCAGGCG
ACTGCGCTGGCAACCGTGCAGCAACTTGATCCGATCTACGTTGATGTGACCCAGTCCAGC
AACGACTTCTGCGCCTGAAACAGGAAGTGGCGAATGGCACGCTGAAACAAGAGAACGGC
AAAGCCAAAGTGTCACTGATCACCAGTGACGGCATTAAAGTTCCTCGCAGGACGGTACGCTG
GAATTCTCTGACGTTACCGTTGATCAGACCACTGGGTCTATCACCTACGCGCTATCTTC
CCGAACCCGGATCACACTCTGCTGCGGGTATGTTCTGTCGCGCACGTCTGGAAGAAGGG
CTTAATCCAAACGCTATTTTAGTCCCGCAACAGGGCGTAACCCGTACGCCGCGTGGCGAT
GCCACCGTACTGGTAGTTGGCGCGGATGACAAAGTGGAAACCCGTCCGATCGTTGCAAGC
CAGGCTATTGGCGATAAGTGGCTGGTGACAGAAGGTCTGAAAGCAGGCGATCGCGTAGTA
ATAAGTGGGCTGCAGAAAGTGCCTGCTGGTGTCCAGGTAAAGCACAAGAAGTTACCGCT
GATAATAACCAGCAAGCCGCAAGCGGTGCTCAGCCTGAACAGTCCAAGTCTTAA

Sequence ID No. 4 – DNA encoding AcrA of E. coli MG1655

MPNFFIDRPIFAWVIAIIIMLAGGLAILKLPVAQYPTIAPPAVTISATYPGADAKTVQDT
VTQVIEQNMNGIDNLMYSSNSDSTGTVQITLTFESGTDADIAQVQVQNKQLAMPLLPQ
EVQQQGVSVKSSSSFLMVGVINTDGMTQEDISDYVAANMKDPISRTSGVGDVQLFGS
QYAMRIWMNPTELTKYQLTPVDVINAIIKAQNAQVAAGQLGGTTPPVKGQQLNASIIAQTRL
TSTDEFGKILLKVNQDGSQVRLRDVAKIELGGENYDVIAKFNGQPASGLGIKLATGANAL
DTATAIRAELKKMEPPFPGMKIVYPYDTPPFVKISIEHVVKTLVEAIIILVFLVMYLFQ
NFRATLIPTIAVPVLLGTFVLAFAFGFSINTLTMFGMVLAIGLLVDDAIVVVENVERVM
TEEGLPPKEATRKSMTQIQGALVGIAMVLSAVFIPMAFFGGSTGAIYRQFSITIVSAMAL
SVLVALILTPALCATMLKPVAKGDHGEKKGFFGWFNRLFDKSTHHTDSVGNILRSTGR
YLLLYLIIIVGMAYLFVRLPSSFLPDEDQGVFLTMVQLPAGATQERTQKVLDEVTDYYLN
KEKANVESVFAVNGFGFAGRGQNTGIAFVSLKDWADRPGEKNKVEAITQRATAAFSQIKD
AMVFAFNLPAIVELGTATGDFELIDQAGLGHEKLTQARNQLFGEVAKYPDLLVGVVRPNG
LEDTPQFKIDIDQEKAQALGVISDINTTLGAAWGGSYVNDIFDRGRVKKVYVMSEAKYR
MLPDDINDWYVRGSDGQMPVPSAFSSSRWEYGSRLERYNGLPSMEILGQAAPGKSTGEA
MAMMEELASKLPSGIGYDWTGMSYQERLSGNQAPALYAIISLIVVFLCLAALYESWSIPFS
VMLVVPLGVIGALLAATFRGLTNDVYFQVGLLTITIGLSAKNAILIVEFAKDLMDKEGKGL
VEATLEAVRMRLRPILMTSLAFMLGVMLPVISSGAGSGAQNAGTGVLGGMVTATVLAIF
FVPVFFVVRRRFSRKSEDIHSHSTEHR

Sequence ID No. 5 – amino acid sequence of AcrB of Salmonella Typhimurium LT2

ATGCCTAATTTCTTTATCGATCGCCCTATATTTGCGTGGGTGATCGCCATCATCATCATG
TTGGCAGGGGGGCTCGCGATCCTCAAATTGCCGGTAGCGCAATATCCGACGATTGCGCCA
CCAGCAGTGACGATCTCCGCAACCTACCCTGGCGCTGATGCGAAAACGGTACAGGATACC
GTCACGCAGGTTATCGAACAGAAATATGAACGGTATCGATAACCTGATGTATATGTCTCTCC
AACAGTGACTCCACGGGGACCGTGCAGATCACGCTGACCTTTGAATCCGGCACCGATGCG
GATATCGCGCAGGTTTACGGTTCAGAAACAAGTTGCAACTGGCAATGCCGTTACTTCCCCAG
GAAGTACAGCAACAGGGCGTGAGCGTTGAGAAGTCCCTCAAGTAGCTTCCTGATGGTAGTG
GGCGTCATTAACACCGACGGCACCATGACCCAGGAGGATATTTCCGATTACGTTGCCGCC
AATATGAAAGATCCGATCAGCCGTACCTCTGGGGTGGGCGACGTCCAGCTGTTTGGTTTCG
CAATATGCGATGCGTATCTGGATGAATCCGACAGAGCTGACCAAATACCAACTGACGCCG
GTCGACGTGATTAACGCGATCAAAGCGCAGAACGCCAGGTGCGGGCAGGTACGCTCGGT
GGTACGCCGCCGGTTAAAGGCCAGCAGCTTAACGCATCGATTATTGCCCAAACGCGTCTG
ACCTCAACGGATGAGTTTGGCAAAATCCTGCTGAAAGTGAATCAGGATGGCTCCCAGGTT
CGTCTGCGGGATGTAGCGAAAATGAGCTTGGCGGCGAGAACTACGACGTCATTGCGAAA
TTTAACGGTCAGCCAGCGTCAGGTCTTGGCATCAAACCTGGCTACCGGCGCCAACGCGCTG
GATACCGCTACCGCTATTCGTGCCGAACCTGAAAAAATGGAACCGTTCTTCCCGCCAGGG
ATGAAAATCGTCTACCCGTATGACACCACGCCGTTCTGTGAAGATCTCTATTTCATGAAGTG
GTAAAAACGCTGGTTCGAAGCGATTATCCTCGTGTTCCTGGTGATGTACCTGTTCTTCGAG
AATCTCCGCGCGAGCTTGATTCCGACTATTGCGGTTCCGGTGGTGTGTTGTTGGGAACCTTT
GCCGTGCTTGCGGCATTCGGTTTCTCGATAAACACGCTGACGATGTTTCGGGATGGTGTCTC
GCCATCGGCTTGCTGGTGGATGACGCCATCGTGGTGGTTCGAGAACGTCGAACGTGTTATG
ACGGAAGAAGGCCTTCCGCCGAAGGAAGCGACGCGCAAATCCATGGGCCAGATTACAGGGC
GCATTGGTGGGTATCGCGATGGTACTGTGCGCGGTATTTATTCCGATGGCCTTCTTTGGC
GGCTCAACCGGGGCAATTTATCGTCAGTTCTCTATCACCATCGTATCGGCGATGGCGCTG
TCGGTGCTGGTTCGCGCTGATCCTGACGCCTGCGCTGTGCGCGACGATGCTCAAACCCGTC
GCCAAAGGCGATCATGGCGAAGGGAAAAAAGGCTTTTTCGGCTGGTTTAACCGCCTGTTT
GATAAGAGCACGCATCACTACACCGATAGCGTAGGCAATATTCTGCGCAGCACCGGGCGT
TATCTGCTGCTCTATCTCATTATCGTCGTCGGTATGGCTTATCTGTTCTGTTCTGCTGCCA
AGCTCTTTCTTGCCGGATGAAGACCAGGGCGTATTCTTGACAATGGTCCAGCTCCCGCGC
GGCGCAACGCAAGAGCGCACGCAAAAAGTGTGCTGGATGAGGTACGGAATTACTATCTGAAC
AAAGAGAAAGCCAACGTTGAATCGGTATTTCGCCGTCACCGCTTCGGTTTTGCAAGGCGC
GGTCAGAATACCGGTATTGCATTCTGTGCTGTGAAAGACTGGGCCGATCGTCCAGGCGAA
AAAAACAAGGTTGAAGCGATTACCCAGCGGGCAACCGCAGCGTTTTTCACAAATTAAAGAT
GCGATGGTCTTTCGCCCTTTAACCTGCCGGCGATCGTTGAGCTGGGCACCGCAACCGGCTTT
GACTTCGAGTTGATTGACCAGGCGGGACTTGGTTCATGAAAACTCACCCAGGCACGTAAT
CAGTTGTTTCGGCGAGGTGGCGAAATATCCTGATCTGCTGGTTCGGCGTTTCGACCTAACGGT
CTGGAAGATACGCCGCAGTTTAAATTCGATATCGACCAGGAAAAAGCTCAGGCGCTGGGC
GTATCTATTAGCGACATTAATACCACGCTGGGCGCAGCATGGGGCGGCAGCTATGTAAAC
GACTTTATCGATCGCGGTCTGTGAAGAAAGTTTACGTGATGTCCGAAGCGAAATACCGC
ATGTTGCCGGATGATATTAACGACTGGTACGTTCTGTGGTAGCGATGGTCAGATGGTGCCA
TTCTCCGCATTCTCCTCTTCCCGCTGGGAATATGGTTTCGCCGCTCTGGAACGCTATAAC
GGTCTGCCTTCGATGGAAATCTGGGGCAGGCGCGCCAGGCAAGAGTACCGGTGAAGCG
ATGGCGATGATGGAAGAACTGGCCAGCAAGCTGCCGTACGGCATTGGGTATGACTGGACC
GGGATGTCTTACCAGGAGCGGTTGTCCGGCAACCGGCCCCCTGCCCTGTATGCTATATCG
CTGATCGTCTGCTTCTCTGTCTGCGGCGATTGTATGAGAGCTGGTCTATCCCGTCTCTCC
GTAATGCTGGTTGTTCCGCTTGGGGTTATCGGCGCGCTGCTGGCTGCGACCTTCCGCGGA
CTGACTAACGACGTTTACTTCCAGGTGGGCCTGCTCACAACCATTTGGGTTGTTCGGCGAAG
AACGCGATACTTATCGTCGAATTCGCCAAAGACTTAATGGATAAAGAAGGGAAAGGCTCTG
GTAGAAGCGACGCTGGAGGCCGTCGGATGCGTTTTCGCCCCGATTCTGATGACCTCGTTA
GCGTTTCATGCTGGGGGTTATGCCGCTGGTTATCAGTTCCGGCGCGGGTTCCGGCGCGCAG
AATGCGGTAGGTACTGGCGTACTGGGCGGGATGGTAACGGCAACCGTACTGGCTATTTTC
TTCGTACCGGTCTTCTTCGTGGTGGTACGCCGCCGCTTTAGCCGTAAAAGCGAAGATATT
GAGCATAGTCATTTCGACAGAACATCGCTGA

Sequence ID No. 6 – DNA encoding AcrB of Salmonella Typhimurium LT2

Figure 1

MPNFFIDRPIFAWVIAIIIMLAGGLAILKLPAQYPTIAPPAVTISASYPGADAKTVQDT
VTQVIEQNMNGIDNLMYMSSNSDSTGTVQITLTFESGTDADIAQVQVQNKQLQAMP LLPQ
EVQQQGVSV EKSSSSFLMVVG VINTDGTMTQEDISDYVAANMKDAISR TSGVGDVQLFGS
QYAMRIWMNPNELNKFQLTPVDVITAIKAQNAQVAAGQLGCTPPVKGQQLNASIIAQTRL
TSTEEFGKILLKVNQDGSRVLLRDVAKIELGGENYDIIAEFNGQPASGLGIK LATGANAL
DTAAAIRAELAKMEPFFPSGLKIVYPYDTTPFVKISIH EVVKTLVEAIIILVFLVMYLFLO
NFRATLIPTIAVPVLLGTFVLA AFGFSINTLTMFGMVLAIGLLVDDAIVVVENVERVM
AEEGLPPKEATR KSMGQIQGALVGIAMVLSAVFVPM AFFGGSTGAIYRQFSITIVSAMAL
SVLVALILTPALCATMLKPIAKGDHGEKKGFFGWFNRMFEKSTHHYTDSVGGILRSTGR
YLVLYLIIVVG MAYLFVRLPSSFLPDEDQGVFMTMVQLPAGATQERTQKVLNEVTHY YLT
KEKNVESVFAVNGFGFAGRGQNTGIAFVSLKDWADRPGEENKVEAITMRATRAFSQIKD
AMVFAFNLPAIVELGTATGDFELIDQAGLGHEKLTQARNQLLAEEAAKHPDMLTSVRPNG
LEDTPQFKIDIDQEKAQALGVSINDINTTLGA AWGGSYVNDFIDRGRVKKVYVMSEAKYR
MLPDDIGDWYVRAADGQMPFSAFSSSRWEYGSPRLERYNGLPSMEILGQAAPGKSTGEA
MELMEQLASKLPTGVGYDWTGMSYQERLSGNQAPSLY AISLIVVFLCLAALYESWSIPFS
VMLVVPLGVIGALLAATFRGLTNDVYFQVGLLT TIGLSAKNAILIVEFAKDLMDKEGKGL
IEATLDAVRMRLRPILMTSLAFILGVMPLVISTGAGSGAQNAVGTGVMGMVTATVLAIF
FVPVFFVVVRRRF SRKNEDIEHSHTVDHH

Sequence ID No. 7 – amino acid sequence of AcrB of E. coli MG1655

Figure 1

ATGCCTAATTTCTTTATCGATCGCCCGATTTTTGCGTGGGTGATCGCQATTATCATCATG
TTGGCAGGGGGGCTGGCGATCCTCAAACCTGCCGGTGGCGCAATATCCTACGATTGCACCG
CCGGCAGTAACGATCTCCGCTCCTACCCCGGCGCTGATGCGAAAAAGTGCAGGACACG
GTGACACAGGTTATCGAACAGAATATGAACGGTATCGATAACCTGATGTACATGTCTCT
AACAGTGACTCCACGGGTACCGTGCAGATCACCCCTGACCTTTGAGTCTGGTACTGATGCG
GATATCGCGCAGGTTACAGTGCAGAACAACTGCAGCTGGCGATGCCGTTGCTGCCGCAA
GAAGTTCAGCAGCAAGGGGTGAGCGTTGAGAAATCATCCAGCAGCTTCCTGATGGTTGTC
GGCGTTATCAACACCGATGGCACCATGACGCAGGAGGATATCTCCGACTACGTGGCGGCG
AATATGAAAGATGCCATCAGCCGTACGTGGGCGTGGGTGATGTTTTCAGTTGTTTCGGTTCA
CAGTACGCGATGCGTATCTGGATGAACCCGAATGAGCTGAACAAATTCAGCTAACGCCG
GTTGATGTCAATTACCGCCATCAAAGCGCAGAACGCCAGGTTGCGGGCGGGTCAGCTCGGT
GGTACGCCGCCGGTGAAAGGCCAACAGCTTAACGCCCTCTATTATTGCTCAGACGCGTCTG
ACCTCTACTGAAGAGTTTCGGCAAAATCCTGCTGAAAGTGAATCAGGATGGTTCCCGCGTG
CTGCTGCGTGACGTGCGGAAGATTGAGCTGGGTGGTGAGAACTACGACATCATCGCAGAG
TTTAACGGCCAACCGGCTTCGGTCTGGGGATCAAGCTGGCGACCGGTGCAAACGCGCTG
GATACCGCTGCGGCAATCCGTGCTGAACTGGCGAAGATGGAACCGTTCTTCCCGTCGGGT
CTGAAAATTTGTTTACCCATACGACACCACGCCGTTTCGTGAAAATCTCTATTACGAAGTG
GTTAAAACGCTGGTCGAAGCGATCATCCTCGTGTTCCTGGTTATGTATCTGTTCCCTGCAG
AACTTCCGCGCGACGTTGATTCCGACCATTTGCCGTACCGGTGGTATTGCTCGGGACCTTT
GCCGTCTTTGCCGCTTTGGCTTCTCGATAAACACGCTAACAAATGTTTCGGGATGGTGCTC
GCCATCGGCCTGTTGGTGGATGACGCCATCGTTGTTGGTAGAAAACGTTGAGCGTGTTATG
GCGGAAGAAGGTTTGGCCGCAAAAGAAGCTACCCGTAAGTCGATGGGGCAGATTACAGGGC
GCTCTGGTCGGTATCGCGATGGTACTGTGCGCGGTATTCGTACCGATGGCCTTCTTTGGC
GGTTCTACTGGTGCTATCTATCGTCAGTTCTCTATTACCATTGTTTCAGCAATGGCGCTG
TCGGTACTGGTGCGTTGATCCTGACTCCAGCTCTTTGTGCCACCATGCTGAAACCGATT
GCCAAAGGCGATCACGGGAAGGTAAAAAAGGCTTCTTCGGCTGGTTTAACCGCATGTTT
GAGAAGAGCACGCACCACTACACCGACAGCGTAGGCGGTATTCGTGCGCAGTACGGGGCGT
TACCTGGTGCTGTATCTGATCATCGTGGTCGGCATGGCCTATCTGTTTCGTGCGTCTGCCA
AGCTCCTTCTTGCCAGATGAGGACCAGGGCGTGTATTATGACCATGGTTTCAGCTGCCAGCA
GGTGCAACGCAGGAACGTACACAGAAAGTGCTCAATGAGGTAACGCATTACTATCTGACC
AAAGAAAAGAACAACGTTGAGTCGGTGTTTCGCCGTTAACGGCTTCGGCTTTGCGGGACGT
GGTCAGAATACCGGTATTGCGTTTCGTTTCTTGAAGGACTGGGCCGATCGTCCGGGCGAA
GAAAACAAAGTTGAAGCGATTACCATGCGTGCAACACGCGCTTTCTCGCAAATCAAAGAT
GCGATGGTTTTTCGCTTTAACCTGCCCGCAATCGTGGAATGGGTACTGCAACCGGCTTT
GACTTTGAGCTGATTGACCAGGCTGGCCTTGGTCACGAAAACTGACTCAGGCGCGTAAC
CAGTTGCTTGCGAAGCAGCGAAGCACCCCTGATATGTTGACCAGCGTACGTCCAAACGGT
CTGGAAGATACCCCGCAGTTTAAAGATTGATATCGACCAGGAAAAAGCGCAGGCGCTGGGT
GTTTCTATCAACGACATTAACACCACTCTGGGCGCTGCATGGGGCGGCAGCTATGTGAAC
GACTTTATCGACCGCGTTCGTGTGAAGAAAGTTTATGTCATGTCAGAAGCGAAATACCGT
ATGCTGCCGGATGATATCGGCGACTGGTATGTTTCGTGCTGCTGATGGTCAGATGGTGCCA
TTCTCGGCGTTCTCCTCTTCTCGTTGGGAGTACGGTTTCGCCGCGTCTGGAACGTTACAA
GGCCTGCCATCCATGGAATCTTAGGCCAGGCGGCACCGGGTAAAAAGTACCGGTGAAGCA
ATGGAGCTGATGGAACAACTGGCGAGCAAACTGCCCTACCGGTGTTGGCTATGACTGGACG
GGGATGTCCTATCAGGAACGTCTCTCCGGCAACCAGGCACCTTCACTGTACGCGATTTCG
TTGATTGTCGTGTTCTGTGTCTGGCGGCGCTGTACGAGAGCTGGTCGATTCCGTTCTCC
GTTATGCTGGTCGTTCCGCTGGGGGTATCGGTGCGTTGCTGGCTGCCACCTTCCGTGGC
CTGACCAATGACGTTTACTTCCAGGTAGGCCTGCTCACAACCATTTGGGTGTCGGCGAAG
AACGCGATCCTTATCGTCAATTCGCCAAAGACTTGATGGATAAAGAAGGTAAAGGTCTG
ATTGAAGCGACGCTTGATGCGGTGCGGATGCGTTTACGTCCGATCCTGATGACCTCGCTG
GCGTTTATCCTCGGCGTTATGCCGCTGGTTATCAGTACTGGTGCTGGTTCCGGCGCGCAG
AACGCAGTAGGTACCGGTGTAATGGGCGGGATGGTGACCGCAACGGTACTGGCAATCTTC
TTCGTTCCGGTATTCCTTTGTGGTTCGCCGCCGCTTTAGCCGCAAGAATGAAGATATC
GAGCACAGCCATACTGTGATCATCATTGA

Sequence ID No. 8 – DNA encoding AcrB of E. coli MG1655

Figure 1

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MQMKLLLPILIGLSLSGFSTLSQAENLMQVYQQARLSNPPELRKSAADRDAAFEKINEARS
 PLLPQLGLGADYTYSNGYRDANGINSNETSASLQLTQTLFDMKWRGLTLQEKAAGIQDV
 TYQTDQQTLILNTANAYFKVLNAIDVLSYTAQKEAIYRQLDQTTQRFNVGLVAITDVQN
 ARAQYDTVLANEVTARNNLDNAVEELRQVTGNYPPELASLNVEHFKTDKPKAVNALLKEA
 ENRNLSLLQARLSQDLAREQIRQAQDGHLPDLNLASTGISDTSYSGSKTNSTQYDDSNM
 GQNKIGLNFSLPLYQGGMVNSQVKQAQYNFVGASEQLESABRSVQTVRSSFNNINASIS
 SINAYKQAVVSAQSSLDAMEAGYSVGTRTIVDVLDAATTTLYDAKQQLANARYTYLINQLN
 IKYALGTLNEQDLLALNSTLGKPIPTSPESVAPETPDQDAADGYNASAPAVQPTAAR
 ANSNNGNPFRRH

Sequence ID No. 9 – amino acid sequence of TolC of Salmonella Typhimurium LT2

ATGCAAATGAAGAAATTGCTCCCCATCCTTATCGGCCTGAGCCTGTCGGGGTTCAGCACA
 CTAAGCCAGGCAGAGAACCTGATGCAAGTTTATCAGCAAGCAGCCTGAGCAACCCGGAA
 TTGCGTAAATCCGCTGCCGATCGCGATGCTGCATTTCGAAAAAATTAACGAAGCGCGTAGT
 CCTTTACTGCGCGCAACTGGGTTTAGGTGCCGACTACACCTACAGCAACGGTTATCGCGAT
 GCGAACGGTATCAACTCCAATGAAACCAGCGCTTCTCTGCAATTAACGCAGACGCTATTT
 GATATGTCGAAATGGCGTGGGCTCACCTGCAAGAAAAGCAGCAGGCATTTCAGGATGTC
 ACCTATCAGACCGATCAGCAGACGCTGATCCTCAATACCGCGAACGCGTATTTAAGGTA
 TTGAACGCTATTGATGTGCTTTTCTATACCCAGGCGCAAAAAGAGGCTATCTACCGTCAG
 TTAGATCAAACGACGCAACGTTTTAACGTGGGTCTGGTCCGCTTACCGACGTCGAAAAAC
 GCCCGTGCAGCAATATGATACCGTACTGGCGAATGAAGTGACCGCCCGCAACAACTGGAT
 AACGCGGTAGAAGAGCTGCGCCAGGTAACCGGCAATTATTACCGGAGCTGGCGTCGCTT
 AACGTCGAGCATTTTAAACCGACAAACCCAAAGCTGTTAATGCGCTGTTGAAGGAAGCG
 GAAAACCGTAACCTGTGCTGTTGTCAGGCGGCTTTAAGTCAGGATCTGGCGCGCGAGCAA
 ATCCGTGAGGCGCAGGATGGTCACCTGCCGACGCTGAATTTAACGGCCTCAACCGGCATT
 TCTGATACCTCTTATAGCGGTTCTAAAACCAACTCCACCCAGTACGACGATAGCAACATG
 GGGCAGAATAAAATCGGCCTTAACCTTCTCCCTGCCGCTGTATCAAGGTGGGATGGTTAAC
 TCGCAGGTAAACAGGCGCAGTATAACTTCGTGCGCGCAAGCGAACAGCTGGAAAGCGCG
 CACCGTAGCGTGGTGCAGACCGTACGTTCTTCTTTAACAATATTAACGCCTCCATCAGC
 AGCATCAACGCGTATAAACAGGCGGTCGTTTCCGCGCAAGGTTCTTTGGATGCCATGGAA
 GCCGGTACTCGGTGCGGTACACGTACCATTTGTTGACGTACTGGATGCCACCACCACTCTG
 TATGATGCCAAGCAGCAACTGGCCAACGCGCGTTATACCTATTTGATTAAATCAGTTAAAT
 ATCAAATATGCGCTCGGTACGCTGAACGAGCAGGATCTGCTCGCGCTTAACAGTACGTTG
 GGTAAACCTATCCCGACGTCGCGGAAAGCGTAGCGCCGGAACGCCAGATCAGGATGCT
 GCCGACAGCGGTTATAATGCTCATAGCGCGCGCCAGCAGTACAGCCGACCGCGCTCGC
 GCCAACAGCAATAACGGCAATCCATTCCGGCATTTGA

Sequence ID No. 10 – DNA encoding TolC of Salmonella Typhimurium LT2

MQMKLLLPILIGLSLSGFSSLSQAENLMQVYQQARLSNPPELRKSAADRDAAFEKINEARS
 PLLPQLGLGADYTYSNGYRDANGINSNATSASLQLTQSI FDMKWRALTLQEKAAGIQDV
 TYQTDQQTLILNTATAYFNVNLNAIDVLSYTAQKEAIYRQLDQTTQRFNVGLVAITDVQN
 ARAQYDTVLANEVTARNNLDNAVEQLRQITGNYPPELAALNVENFKTDKPPVNALLKEA
 EKRNLSSLLQARLSQDLAREQIRQAQDGHLPDLNLASTGISDTSYSGSKTRGAAGTQYDD
 SNMGQNKVGLSFLPIYQGGMVNSQVKQAQYNFVGASEQLESABRSVQTVRSSFNNININA
 SISSINAYKQAVVSAQSSLDAMEAGYSVGTRTIVDVLDAATTTLYNAKQELANARYNYLIN
 QLNKISALGTLNEQDLLALNNALSKPVSTNPENVAPQTPEQNAIADGYAPDSPAPVVQQT
 SARTTTSNHNPFRN

Sequence ID No. 11 – amino acid sequence of TolC of E. coli MG1655

Figure 1

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ATGCAAATGAAGAAATTGCTCCCATTCCTTATCGGCCTGAGCCTTTCTGGGTTTCAGTTTCG
TTGAGCCAGGCCGAGAACCTGATGCAAGTTTATCAGCAAGCACGCCCTAGTAACCCGGAA
TTGCGTAAAGTCTGCCGCCGATCGTGATGCTGCCTTTGAAAAAATTAATGAAGCGCGCAGT
CCATTACTGCCACAGCTAGGTTTAGGTGCAGATTACACCTATAGCAACGGCTACCGCGAC
GCGAACGGCATCAACTCTAACGCGACCAGTGCGTCTTGCAGTTAACTCAATCCATTTTT
GATATGTCGAAATGGCGTGCGTTAACGCTGCAGGAAAAAGCAGCAGGGATTTCAGGACGTC
ACGTATCAGACCGATCAGCAAACCTTGATCCTCAACACCGCGACCGCTTATTTCAACGTG
TTGAATGCTATTGACGTTCTTTTCCTATACACAGGCACAAAAAGAAGCGATCTACCGTCAA
TTAGATCAAACCACCCAACGTTTTTAACGTGGGCCTGGTAGCGATCACCGACGTGCAGAAC
GCCCGCGCACAGTACGATACCGTGCTGGCGAACGAAGTGACCGCACGTAATAACCTTGAT
AACGCGGTAGAGCAGCTGCGCCAGATCACCGGTAACCTACTATCCGGAACGGCTGCGCTG
AATGTCGAAAACCTTTAAACCCGACAAACACAGCCGGTTAACGCGCTGCTGAAAGAAGCC
GAAAAACGCAACCTGTGCTGTTACAGGCACGCTTGAGCCAGGACCTGGCGCGCGAGCAA
ATTGCGCCAGCGCAGGATGGTCACTTACCGACTCTGGATTAAACGGCTTCTACCGGGATT
TCTGACACCTCTTATAGCGGTTCGAAAACCCGTGGTGCCGCTGGTACCCAGTATGACGAT
AGCAATATGGGCCAGAACAAAGTTGGCCTGAGCTTCTCGCTGCCGATTTATCAGGGCGGA
ATGGTTAACTCGCAGGTGAAACAGGCACAGTACAACCTTTGTCGGTGCCAGCGAGCAACTG
GAAAGTGCCCATCGTAGCGTCGTGCAGACCGTGCGTTCTCCTTCAACAACATTAATGCA
TCTATCAGTAGCATTAAACGCCTACAAACAAGCCGTAGTTTTCCGCTCAAAGCTCATTAGAC
GCGATGGAAGCGGGCTACTCGGTGCGTACGCGTACCATTGTTGATGTGTTGGATGCGACC
ACCACGTTGTACAACGCCAAGCAAGAGCTGGCGAATGCGCGTTATAACTACCTGATTAAT
CAGCTGAATATTAAGTCAGCTCTGGGTACGTTGAACGAGCAGGATCTGCTGGCACTGAAC
AATGCGCTGAGCAAACCGGTTTCCACTAATCCGAAAAACGTTGCACCGCAAACGCCGGAA
CAGAAATGCTATTGCTGATGGTTATGCGCCTGATAGCCCCGCCACCGTTCGTTTCAGCAAACA
TCCGCACGCACTACCACCAGTAACGGTCATAACCCTTTCCGTAACCTGA

Sequence ID No. 12 – DNA encoding TolC of E. coli MG1655

MKLFQKNTILALGVVLLLTACSKKEAPKIQMPPQPVTTMSAKSEDLPLSFYPAKLVS DY
DVI IKPQVSGVIENKLFKAGDKVKKGT LFIIEQDKFKASVDSAYGQALMAKATFENASK
DFNRSKALFSKSAISQKEYDSSLATFNNSKASLASARAQLANARIDL DHT EIKAPFDGTI
GDALVNIGDYVSASTTELVRVTNLNPIYADFFISD TDK LNLVRNTQSGKWDLDSIHANLN
LNGETVQGLYFIDSVIDANSGTVKAKAIFDNNNSTLLPGAFATITSEGF IQKNGFKVPQ
IAVKQNQNDVYVLLVKNKVEKSSVHISYQNNEYAII DKGLQNGDKIILDNFKKIQVGSE
VKEIGA Q

Sequence ID No. 13 – amino acid sequence of CmeA of C. jejuni 11168

Figure 1

ATGAAATTATTTCAAAAAAATACTATTTTAGCTTTAGGTGTTGTGCTTTTACTCACTGCT
TGCAGCAAAGAAGAAGCACCAAAAAATACAAATGCCGCCTCAACCTGTAACAACCATGAGT
GCTAAATCTGAAGATTTACCACCTTAGTTTACTTACCCTGCTAAACTTGTCACTGATTAT
GATGTCATTATAAAACCTCAAGTTAGTGGCGTAATAGAAAAATAAACTTTTAAAGCTGGA
GATAAAGTAAAAAAGGACAAACATTATTTATTATAGAACAAAGATAAAATTTAAAGCTAGT
GTTGATTCCGCTTACCGACAAGCTTTGATGGCTAAAGCAACTTTCGAAAAATGCAAGCAAG
GATTTTAATCGTTCTAAAGCTCTTTTAGTAAAAAGTGCAATCTCTCAAAAGGAATACGAC
TCTTCTCTTGCTACATTTAACAATTCAAAAGCTAGTCTAGCAAGTGCTAGAGCACAGCTT
GCAAATGCAAGAATTGATCTAGATCATACCGAAATAAAAGCTCCTTTTGATGGTACTATA
GGAGATGCTTTAGTTAATATAGGAGATTATGTAAGTGCTTCAACAAGTGAAGTACTAGTTA
GTTACAAATTTAAATCCTATTTACGCAGATTTCTTTATTTTCAGATACAGATAAACTAAAT
TTAGTCCGCAATACTCAAAGTGGAAAAATGGGATTTAGACAGCATTTCATGCAAATTTAAAT
CTTAATGGAGAAACCGTTCAAGGCAAACCTTTATTTTATTGATTCTGTTATAGATGCTAAT
AGTGGAAACAGTAAAAGCCAAAGCTATATTTGACAACAACAACCTCAACACTTTTACCAGGT
GCTTTTGCACAATTTACTTTCAGAAAGTTTATACAAAAAATGGCTTTAAAGTGCCTCAA
ATAGCTGTTAAACAAAATCAAAATGATGTTTATGTTCTTCTGTTAAAAATGGAAAAAGTA
GAAAAATCTTCTGTACATATAAGCTACCAAAACAATGAATATGCCATTATTGACAAAGGA
TTGCAAAATGGCGATAAAATCATTTTAGATAACTTTAAAAAAATTCAGTTGGTAGCGAA
GTTAAAGAAATTGGAGCACAAATAA

Sequence ID No. 14 – DNA encoding CmeA of *C. jejuni* 11168

MFSKFFIERPVFASVVAIIISLAGAIGLTNLPIEQYPSLTPPTVKVSATYTGADAQTIAS
TVASPIEDAINGADNMIYMDSTSSSSGTMSTLVYFDIGTDPDQATIDVNNRISAATAKMP
DAVKKLGVTVRKTSSTTLAAISMYSSDGSMASVDVYNYITLNVLDLKRVPVGDANAIG
NRNYSRLRIWLKPDLLNKFGITATDVISAVNDQNAQYATGKIGEEPVTQKSPYVYSITMQG
RLQNPSEFENIILRTNNDGSFLRLKDVADEIGSQYSSQGRNLNGNDAVPIMINLQSGAN
ALHTAELVQAKMQELSKNFPKGLTYKIPYDTTKFVIESIKEVVKTFVEALILVIVMYMF
LKNFRATLIPMIAPVSVLLGTFAGLYVLGFSINLLTLFALILAIIGIVVDDAIIVVENIDR
ILHENEQISVKDAAIQAMQEVSSPVISIVLVLCVFPVPSFISGFVGEIQRQFALTALIS
VTISGFVALTLTPSLCALFLRRNEGEPFKFVKKFNDFFDWSTSVFSAGVAYILKRTIRFV
LIFCIMLGAIFYLYKAVPNSLVPEEDQGLMISIIINLPSASALHRTISEVDHISQEVKLTN
GVKDAMAMIGFDLFTSSLKENAAAMFIGLQDWKDRNVSADQIIAELNKKFAFDRNASSVF
IGLEPIPGLSITGGFEMYVQNKSGKSYDEIQKDVNKLAVANQRKELSRVRTTLDTTFPQ
YKLIIDRDKLKHYNLNMQDVFNMTNATIGTYVNDFSMLGKNFQVNIRAKGDFRNTQDAL
KNIFVRSNDGKMIPLDSFLTLQRSSGPDDVKRFNLFPAAQVQGPAPGYTSGQAIEAIAQ
VAKETLGDDYSIAWGSAYQEVSSKGTASYAFALGMVVFVFLILAAQYERWLIPLAVVTAV
PFAVFGSFLLVYLRGFSNDIYFQTGLLLLLIGLSAKNAILLIVEFAMEERFKKGKGVFEAAV
AAAKLRFRIIMTSLAFTFGVLPIMIFATGAGSASRHSLSLTGLIGGMIAASTLAIFFVPLF
FYLLENFNEWLDKKRGKVHE

Sequence ID No. 15 – amino acid sequence of CmeB of *C. jejuni* 11168

ATGTTTTCTAAATTTTTTATCGAAAGACCTGTTTTTGCCTTCAGTTGTTGCAATTATCATTT
TCTTTAGCTGGAGCTATAGGTCTTACAAATTTACCTATAGAACAATATCCTTCTTTAACC
CCTCCTACAGTTAAAGTAAGTGCAACTTACACAGGAGCTGATGCACAAACCATTTGCTTCA
ACAGTTGCAAGTCCTATCGAAGATGCAATCAATGGCGCAGATAATATGATTTACATGGAT
TCAACTTCAAGTTCTTCAGGAACATAGAGTTTACTGTTTATTTTGATATCGGTACAGAT
CCTGATCAAGCCACCATAGATGTTAATAATAGAATTTTACGCCGCAACTGCAAAAATGCCA
GATGCAGTTAAAAAACCTTGGAGTAACAGTTAGAAAAACCTCCTCAACAACACTTGCTGCA
ATTTCTATGTATTCAAGTGATGGCTCAATGAGTGCAAGTAGATGTATATAACTATATCACT
TTAAATGTTTTAGATGAGTTAAAAAGGGTTCCAGGAGTTGGAGATGCAAACGCTATAGGA
AATCGTAATTATTTCTTTAAGAATTTGGCTCAAGCCTGATTTATTAATAAAATTTGGAATC
ACAGCTAGATGATAATTTCTGCGGTAAATGACCAAAATGCCCAATACGCAACTGGTAA
ATTGGCGAAGAACCCTGTAACCCAAAAAATCCCCTTATGTTTATTCAATCACTATGCAAGGA
AGACTACAAAATCCTAGTGAATTTGAAAAACATTATTTTAAGAACAATAATGATGGATCA
TTTTTAAGACTTAAAGATGTAGCTGATGTGGAATAGGATCACAACAATACAGCTCACAA
GGACGATTAAATGGCAATGATGCAGTTCCAATTATGATCAATCTTCAATCAGGGGCAAA
GCATTGCATACAGCAGAACTTGTTCAGCCAAAATGCAAGAACTTTCAAAAATTTTCCA
AAAGGTTTAAACATATAAAATTCCTTACGATACAACAAAATTTGTAATAGAATCAATCAAA
GAAGTTGTTAAAACCTTTTGTGGAAGCTCTAATTTTAGTTATCATTTGTTATGTATATGTTT
TTAAAGAATTTCCGTGCAACACTTATTCCTATGATAGCTGTACCTGTTTCATTTGTTAGGA
ACTTTTGCTGGACTTTATGTTTTAGGTTTTAGTATTAACCTTGCTTACGCTTTTTGCTTTA
ATTTTAGCCATAGGGATCGTTGTAGATGATGCGATTATAGTTGTGGAATAATGATAGG
ATTTTACATGAAATGAGCAAATAAGCGTAAAGATGCTGCTATCCTCAAGCGATGCAAGAA
GTTAGCTCTCCGGTTATTTCAATTGTTCTTGTGCTTTGTGCTGTTTTGTACCAGTTTCT
TTTATATCAGGTTTTTGTGAGAGATTCAAAGACAATTTGCTCTTACCTTAGCTATATCT
GTAACCATATCAGGTTTTGTTGCTCTTACCTTAACACCTTCTTTATGTGCACTCTTTTTG
CGACGCAATGAAGGAGAGCCATTTAAATTTGTAAAGAAATTTAATGATTTTTTTGATTGG
AGTACCTCTGTATTTAGCGCAGGAGTAGCATATATCCTAAAAAGAACCATTTCGTTTTGTT
TTAATTTTTTTGTATTATGCTTGGAGCAATTTTTTATCTCTATAAAGCAGTACCAAATTC
TTAGTTCTGAAGAAGATCAAGGTTTGATGATTAGCATTTAATCTTCCTTCAGCTTCA
GCACTCCATAGAACAATTTAGAGGTTGATCACATAAGCCAAGAAGTTTTAAAACTAAT
GGAGTTAAAGATGCAATGGCTATGATAGGATTGATCTTTTACAAGTTCACTCAAAGAA
AATGCCGCTGCAATGTTTATAGGTTTGCAAGATTGGAAGATAGAAATGTAAGCGCTGAT
CAAAATTATAGCCGAACCTTAATAAAAAAATTTGCTTTTGATCGCAATGCTTCAAGTGATTT
ATAGGCTTACCTCTATACCTGGATTAAAGTATCACAGGCGGTTTTGAAATGTATGTTCAA
AACAAAAGCGGAAAAAGCTATGATGAAATACAAAAGATGTAAATAAATCTGTTGCTGTG
GCCAACCAAGAAAAAGAGCTATCAAGAGTAAGAACAACCTTGATACAACCTTCCCTCAA
TACAAGCTTATAATTGATAGAGATAAATTAACACACTACAATCTTAACATGCAAGATGTT
TTTAACACGATGAATGCAACTATAGGCACTTATTATGTTAATGATTTTTCTATGCTAGGT
AAAAATTTCCAAGTAAATATCCGCGCAAAAGGTGATTTTAGAAATACACAAGATGCGTTA
AAAAATATTTTGTAAAGATCAAAATGATGGAATAATGATACCACTTGATTCTTTCTTAACT
TTACAAAGAAGTTCAAGGCTGATGATGTAAACGATTCAACCTTTTCCAGCAGCACAA
GTTCAAGGTCAACCCGACCAAGGTTTACTTCAAGCCAAAGCTATAGAAGCGATTGCTCAA
GTAGCAAAAAGAACTTTAGGGGATGATTATTCGATAGCTTGGAGTGGATCAGCTTATCAA
GAAGTTTCTAGTAAAGGAACAGCAAGCTATGCTTTTGTCTTTAGGCATGGTATTTGTCTTT
TTAATCCTAGCTGCCAATATGAAAGATGGCTTATACCTTTAGCAGTTGTAACAGCTGTG
CCTTTTGCGGTATTTGGATCATTTTTTATTGGTATATTTAAGAGGATTTAGCAATGATATA
TATTTTCAAACAGGACTTTTGCTCTTGATTGGACTTTCAGCTAAAAATGCTATCTTGATC
GTAGAATTTGCAATGGAAGAGCGCTTTAAAAAAGGTAAAGGGGTTTTTGAAGCAGCTGTT
GCAGCAGCAAACTTCGTTTTCGTCCTATCATAATGACTTCTTTGGCATTTACTTTTGGG
GTCTTGCCAATGATTTTCGCAACAGGAGCAGGAAGTGCTTCAAGACACTCTTTAGGAACA
GGGCTTATTGGTGGAATGATCGCAGCATCAACTTTAGCGATATCTTTGTGCCCTTATTT
TTCTATCTTTTAGAAAAATTTAATGAATGGCTAGATAAAAAAAGAGGTAAGGTTTCATGAA
TAA

Sequence ID No. 16 – DNA encoding CmeB of *C. jejuni* 11168

Figure 1

10/12

MNKIIISISAIASF'TLLISACSLSPNLNIPeANYSIDNKLGALESWEKENSSITKNWWKDF
 DDENLNKVVLDLALKNNNDLKLAFIHMEQAAAQLGIDFSSLLPKFDGSASGSRAKTAINAP
 SNRTGEVSYGNDFKMGLNLSYEIDLWGKYRDTYRASKSGFKASEYDYEAAARLSVISNTVQ
 TYFNLANAYENENALKETYESAKEIYRINDEKFQVGAVGEYELAQARANLESMALQYNEA
 KLNKENYLNKALKIILTSNDLNDILYKNQSYQVFNLFKEFDIPTGISSTILLQRPDIGSSLEK
 LTQQNYLVGVARTAF'LPSLSLTGLLGFESGDLDTLVKGGSKTWNIGGNFTLPIFWHGEIY
 QNVNLAKLNKDEAFVNYQNTLITAFGEIRYALVARKTIRLQYDNAQASEQSYKRIYELAK
 ERYDIGEMSLQDYLEARQNWLNAAVAFNNTKYSYANSIVDVIKAFGGGFQSEDTSKNIK
 EESKNLDMSFRE

Sequence ID No. 17 – amino acid sequence of CmeC of *C. jejuni* 11168

ATGAATAAAATAATTTCAATTAGCGCTATAGCAAGTTTTACTCTTTTGATTTCAGCTTGC
 TCTTTAAGTCCAAATTTAAATATTCCCGAAGCAAACCTATAGCATTGATAATAAGCTTGGA
 GCCTTATCCTGGGAAAAAGAAAACAATAGCTCTATCACAAAAAATGGTGGAAAGACTTT
 GATGATGAAAAATTAAATAAAGTGGTTGATTAGCACTTAAAAATAATAATGATTTAAAA
 CTTGCTTTTCATACACATGGAACAAGCTGCTGCTCAATTAGGTATAGATTTTAGCAGTTTG
 TTGCCAAAAATTGATGGTAGTGCAAGCGGAAGTCGTGCAAAAAACAGCTATAAATGCTCCA
 AGCAATCGAAGTGGGGAAGTAAGTTACGGTAATGATTTTAAAAATGGGACTTAATTTAAGC
 TATGAAATCGATCTTTGGGGAAAAATATCGCGATACATATCGCGCCTCAAAATCAGGCTTT
 AAAGCAAGTGAGTATGATTATGAAGCTGCAAGACTTTCTGTTATTTCAAATACAGTTCAA
 ACTTATTTTAAATCTTGCAAAATGCTTATGAAAAATGCTCTTAAAGAAACCTATGAA
 TCGGCAAAAGAAATTTATAGGATTAATGATGAAAAATTTCAAGTTGGTGCTGTAGGTGAA
 TATGAAC'TTGCTCAAGCAAGAGCCAAC'TTAGAAAATATGGCTTTGCAATATAATGAAGCA
 AAGTTAAATAAAGAAAATTACCTTAAAGCTTTAAAAATTTTAACTTCAAATGATTTAAAT
 GACATAC'TTTACAAAAATCAAAGCTATCAAGTTTTTAACTTAAAGAATTTGACATTCCA
 ACTGGAATTTCAAGTACCATCTTGCTTCAACGTCAGATTTGGCTCTTCTTTAGAAAAA
 TTAAC'TCAGCAAAATATCTTGTGGAGTAGCCCGCACGGCTTTCTTACCTAGCCTTTCT
 TTAACAGGATTAATGGGATTTGAAAGTGGGGATTTAGATACCTTGGTTAAAGGAGGTCT
 AAGACTTGGAAATAGGTGGAAACTTTACTCTGCCTATTTTTCATTTGGGGTGAAATTTAC
 CAAAATGTAAATTTAGCTAAGCTTAATAAAGATGAAGCTTTTGTAAATATCAAAATACT
 TTGATTACTGCTTTTGGAGAAATTCGCTATGCTTTAGTAGCTAGAAAACTATACGCTTA
 CAATACGATAATGCACAAGCAAGCGAACAATCTTACAAAAGAATCTATGAAATTGCTAAA
 GAACGCTATGATATAGGAGAAATGTCTTTGCAAGATTATTTAGAAGCACGTCAAAATTGG
 CTTAATGCTGCCGTTGCTTTTAATAATACTAAATATTCTTATGCCAATTCATAGTAGAT
 GTAATCAAAGCATTGGTGGAGGATTTGAGCAAAGTGAAGATACGAGTAAAAATATAAAA
 GAAGAATCAAAAATTTAGATATGTCTTTTAGAGAATAG

Sequence ID No. 18 – DNA encoding CmeC of *C. jejuni* 11168

MQRTPAMRVLVPALLVAISALSGCGKSEAPPPAQTPVEGVITLLEAQTVTLNTELPGRITNA
 FRIAEVRPQVNGIILKRLFKEGSDVKAGQQLYQIDPATYEADYQSAQANLASTQEQAQRY
 KLLVADQAVSKQQYADANAAYLQSKAAVEQARINLRYTKVLSPISGRIGRSVTEGALVT
 NGQANAMATVQQLDPIYVDVTQPS'TALLRLRRELASQLERAGDNAAKVSLKLEDGSQYP
 LEGRLEFSEVSDEGTGSVTIRAVFPNPNNELLPGMFVHAQLQEGVKQKAILAPQQGVTR
 DLKGQATALVVNAQNKVELRVIKADRVIGDKWLVTEGLNAGDKIITEGLQFVQPGVEVK
 VPAKNVSAQKADAAPAKTDSKG

Sequence ID No. 19 – amino acid sequence of MexA of *P. aeruginosa* PA01

Figure 1

11/12

ATGCAACGAACGCCAGCCATGCGTGTA TGGTTCGGGCCCTGCTGGTTCGCGATTTTCGGCC
CTTTCGGGTGCGGAAAAAGCGAGGCGCCGCGCCGCGCGCAAACGCCGGAGGTCGGGATC
GTGACCCCTGGAAGCGCAGACGGTGACCCCTGAATACCGAGCTGCCGGGCCGGACCAATGCG
TTCCGCGATCGCCGAGGTGCGTCCCCAGGTGAACGGCATCATCCTCAAGCGCCTGTTCAAG
GAAGGCAGCGACGTCAAGGCCGGGCAGCAGCTCTACCAGATCGACCCCGCCACCTACGAG
GCCGACTACCAGAGCGCCCAGGCCAACCTGGCTTCGACCCAGGAACAGGCCCAGCGCTAC
AAGCTGCTGGTTCGCCGACCAGGCCGTGAGCAAGCAGCAGTACGCCGACGCCAATGCCGCC
TACCTGCAGTCCAAGGCGGCGGTGGAGCAGGCGCGGATCAACCTGCGCTACACCAAGGTG
CTGTGCGCGATCTCCGGCCGCATCGGCCGTTCCGCGGTGACCGAAGGCGCCCTGGTGACC
AACGGCCAGGCCAACGCGATGGCCACCGTGCAACAGCTCGACCCGATCTACGTCGACGTC
ACCCAGCCGTCCACCGCCCTGCTGCGCCTGCGCCGCGAACTGGCCAGCGGCCAGTTGGAG
CGCGCCGGCGACAACGCGCGAAGGTCTCCCTGAAGCTGGAGGACGGTAGCCAATACCCG
CTGGAAGGTTCGCTCGAATTCTCCGAGGTTTCCGTCGACGAAGGCACCGGCTCGGTACCC
ATCCGCGCCGTGTTCCCAACCCGAACAACGAGCTGCTGCCCGGCATGTTTCGTTACGCG
CAGTTGCAAGGAAGGCGTCAAGCAGAAGGCCATCCTCGCTCCGCAGCAAGGCGTGACCCGC
GACCTCAAGGGCCAGGCTACCGCGCTGGTGGTGAACGCGCAGAACAGGTGAGCTGCGG
GTGATCAAGGCCGACCGGTGATCGGCGACAAGTGGCTGGTTACCGAAGGCCTGAACGCC
GGCGACAAGATCATTACCGAAGGCCTGCAGTTCGTGCAGCCGGGTGTCGAGGTGAAGACC
GTGCCGGCGAAGAATGTCGCGTCCGCGCAGAAGGCCGACGCCGCTCCGGCGAAAACCGAC
AGCAAGGGCTGA

Sequence ID No. 20 – DNA encoding MexA of *P. aeruginosa* PA01

MSKFFIDRPIFAWVIALVIMLAGGLSILSLPVNQYPAIAPPAIAVQVSYPGASAETVQDT
VVQVIEQQMNGIDNLYISSSNSDGSMTITVTTFEQGTDPDIAQVQVQNKQLLATPLLPQ
EVQRQGI RVTKAVKNFLMVGVVSTDGSMTKEDLSNYIVSNIQDPLSRTKGVGDFQVFGS
QYSMRIWLDPAKLNSYQLTPGDVSSAIQAQNVQISSGQLGGLPAVKGQQLNATIIGKTRL
QTAEQFENILLKVNPDGSQVRLKDVADVGLGGQDYSINAQFNGSPASGIAIKLATGANAL
DTAKAIRQTIANLEPFMPQGMKVVPYDTPVVSASIEHVVKTLGEAILLVFLVMYLFLLQ
NFRATLIPTIAPVPVLLGTFGVLAAGFSINTLTMTFGMVLAIGLLVDDAIVVVENVERVM
AEEGLSPREAARKSMGQIQGALVGIAMVLSAVFLPMAFFGGSTGVIYRQFSITIVSAMAL
SVIVALILTPALCATMLKPIEKGDHGEHKGGFFGWFNRMFLSTTHGYERGVASILKHRAP
YLLIYVVIVAGMIWMFTRIPTAFLPDEDQGVLFQVQTPPGSSAERTQVVVDSMREYLLE
KESSSVSVFTVTGTFNAGRGQSSGMAFIMLKPEERPGGENSVFELAKRAQMHHFFSKD
AMVFAFAPPSVLELGNATGFDLFLQDQAGVGHEVLLQARNKFLMLAAQNPAQVRPNMG
SDEPQYKLEIDDEKASALGVSLADINSTVSIANGSSYVNDFIDRGRVKRVYLQGRPDARM
NPDDLKWKYVRNDKGEMVFPNATGKWEYGSPLERYNGVPAMEILGEPAPGLSSGDAM
AAVEEIVKQLPKGVGYSWTGLSYEERLSGSQAPALYALSLLVVFLCLAALYESWSIPFSV
MLVVPLGVIGALLATSMRGLSNDVFFQVGLLTITIGLSAKNAILIVEFAKELHEQKGIVE
AAIEACRMRLRPIVMTSLAFILGVVPLAISTGAGSGSQHAIGTGVIGMVTATVLAIFWV
PLFYVAVSTLFKDEASKQQASVEKGQ

Sequence ID No. 21 – amino acid sequence of MexB of *P. aeruginosa* PA01

Figure 1

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CGGGCGGTTCGAGGAGATCGTCAAGCAATTGCCGAAAGGCGTTGGCTACTCCTGGACCGGC
CTGTCTACGAGGAGCGCTTGTCCGGCTCGCAGGCGCCGGCGCTGTATGCGCTGTGCGTG
CTGGTGGTGTCTCTCTGCTTGGCGGCCCTGTACGAAAGCTGGTTCGATTCCGTTCTCGGTG
ATGCTGGTGGTGGCGTTGGGCGTGATCGGTGCGCTGCTGGCGACGTCCATGCGCGGCCTG
TCCAACGACGTGTTCTTCCAGGTGGGCCTGTTGACGACCATCGGCCTGTGCGCGAAGAAC
GCCATTCTCATCGTGGAGTTTCGCCAAGGAGCTGCACGAGCAGGGCAAGGGCATCGTTCGAG
GCGGCCATCGAAGCCTGCCGCATGCGTCTGCGGCCGATCGTGATGACCTCCCTGGCGTTC
ATCCTCGGCGTGGTCCCGCTGGCGATCTCCACCGGCGCCGGCTCGGGCAGCCAGCATGCG
ATCGGTACCGGCGTGATCGGCGGCATGGTCACTGCGACCGTCTTGGCGATCTTCTGGGTA
CCGCTGTTCTACGTGGCGGTGACGACGCTGTTCAAGGACGAGGCGTCCAAGCAGCAGGCG
TCCGTCGAAAAGGGGCAATGA

Sequence ID No. 22 – DNA encoding MexB of *P. aeruginosa* PA01

MKRSFLSLAVAAVVLSGCSLIPDYQRPEAPVAAAYPQGQAYGQNTGAAAVPAADIGWREF
FRDPQLQQLIGVALENNRDLRVAALNVEAFRAQYRIQRADLFPRIGVDGSGTRQRLPGDL
STTGSPAISSQYGVTLGTTAWELDLFGRRLSLRDQALEQYLATEQAQRSAQTTLVASVAT
AYLTLKADQAQLQTLTKDLGTQKSFDLTQRSYDVGVASALDLRQAQTAVEGARATLAQY
TRLVAQDQNALVLLLGSIPANLPQGLGLDQTLTTEVPAGLPDILLQRRPDILEAEHQLM
AANASIGAARAAFFPSISLTANAGTMSRQLSGLFDAGSGSWLFQPSINLPIFTAGSLRAS
LDYAKIQKDINVAQYEKAIQTAFQEVADGLAARGTFTEQLQAQRDLVKASDEYYQLADKR
YRTGVDNYLTLLDAQRSFLTAAQQQLITDRLNQLTSEVNLYKALGGGWNQQTVTQQQTAKK
EDPQA

Sequence ID No. 23 – amino acid sequence of OprM of *P. aeruginosa* PA01

ATGAAACGGTCTCTCTCTTCCCTGGCGGTAGCCGCTGTCTGTTCTGTCCGGCTGCTCGCTG
ATCCCCGACTACCAGCGCCCCGAGGCGCCGCTAGCCGCGGCCCTACCCGCAAGGGCAGGCC
TACGGGCAGAACACCGGCGCGGCGGCCGTTCCGGCGCCGACATCGGCTGGCGCGAGTTC
TTCGCGGACCCGAGTTGCAGCAACTGATCGGCGTGGCGCTGGAAAACAACCGCGACCTG
CGGGTCGCCGCGCTGAACGTCGAGGCCCTCCGGGCGCAGTACCGCATCCAGCGGGCCGAC
CTGTTTCCCGCGGATCGGCGTGGACGGTAGCGGCACCCGCCAGCGTTTGGCCGGGCGACCTG
TCGACCACCGGCAGTCCGGCGATTTCAGCCAGTACGGGGTGACCTGGGCACTACCGCC
TGGGAATCGATCTCTTCGGCCGCTGCGCAGCCTGCGCGACCAGGCCCTGGAGCAGTAC
CTGGCGACCCGAACAGGCGCAGCGCAGCGCGCAGACCACCTGGTGGCCAGCGTGGCGACC
GCCTACCTGACGCTGAAGGCCGACAGGCGCAGTTGCAGCTGACCAAGGACACCTGGGC
ACCTACCAGAAGAGTTTCGACCTGACCCAGCGCAGCTACGACGTCGGCGTCGCCTCCGCG
CTCGACCTGCGCCAGGCGCAGACCGCCGTGGAAGGCGCCCGCGGACCTGGGCGCAGTAC
ACCCGCTGGTAGCCAGGACAGAAATGCGCTGGTCTGCTGCTGGGCTCCGGGATCCCG
GCGAACCTGCCGCAAGGCCTGGGCTGGACCAGACCTGCTGACCGAAGTGCCGGCGGGT
CTGCCGTTCGACCTGCTGCAACGGCGCCCGGACATCCTCGAGGCCGAGCACCAGCTCATG
GCTGCCAACGCCAGCATCGGCGCCGCGCGCGGCGTTCCTCCCGAGCATCAGCCTGACC
GCCAACGCCGGCACCATGAGCCGCCAACTGTCCGGCCTGTTTCGACGCCGGTTTCGGTTCC
TGGTTGTTCCAGCCGTCGATCAACCTGCCGATCTTACCGCCGCGAGCCTGCGTGCCAGC
CTGGACTACGCGAAGATCCAGAAGGACATCAACGTCGCGCAGTACGAGAAGGCGATCCAG
ACGGCGTTCCAGGAAGTCGCCGACGGCCTGGCCGCGCGCGGTACCTTACCGAGCAGTTG
CAGGCGCAGCGCGATCTGGTCAAGGCCAGCGACGAGTACTACCAGCTCGCCGACAAGCGC
TATCGCACGGGGGTGGACAACTACCTGACCCCTGCTCGACGCGCAACGCTCGCTGTTTACC
GCGCAGCAGCAACTGATCACCGACCGCCTCAATCAGCTGACCAGCGAGGTCAACCTGTAC
AAGGCCCTCGGCGGCGGCTGGAACCAGCAGACCGTGACCCAGCAGACCGCGAAGAAG
GAAGATCCCCAGGCTTGA

Sequence ID No. 24 – DNA encoding OprM of *P. aeruginosa*

Figure 1