Review

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Treatment of plague: promising alternatives to antibiotics

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Plague still poses a significant threat to human health, and interest has been renewed recently in the possible use of *Yersinia pestis* as a biological weapon by terrorists. The septicaemic and pneumonic forms are always lethal if untreated. Attempts to treat this deadly disease date back to the era of global pandemics, when various methods were explored. The successful isolation of the plague pathogen led to the beginning of more scientific approaches to the treatment and cure of plague. This subsequently led to specific antibiotic prophylaxis and therapy for *Y. pestis*. The use of antibiotics such as tetracycline and streptomycin for the treatment of plague has been embraced by the World Health Organization Expert Committee on Plague as the 'gold standard' treatment. However, concerns regarding the development of antibiotic-resistant *Y. pestis* strains have led to the exploration of alternatives to antibiotics. Several investigators have looked into the use of alternatives, such as immunotherapy, non-pathogen-specific immunomodulatory therapy, phage therapy, bacteriocin therapy, and treatment with inhibitors of virulence factors. The alternative therapies reported in this review should be further investigated by comprehensive studies of their clinical application for the treatment of plague.

Overview

As a Gram-negative bacterium, the causative agent of plague, *Yersinia pestis* (Yersin, 1894), has been the cause of three pandemics (Achtman *et al.*, 1999; Drancourt *et al.*, 2004; Guiyoule *et al.*, 1994; Raoult *et al.*, 2000), and has led to the deaths of millions of people, the devastation of cities and villages, and the collapse of governments and civilizations (Zietz & Dunkelberg, 2004). At the present time, the circulation of *Y. pestis* has been detected within the populations of more than 200 species of wild rodent inhabiting natural plague foci on all continents, except for Australia and Antarctica, and the transmission of plague is provided for by a minimum of 80 species of flea. Plague epizootics, during which the pathogen spreads through new territories, alternate with a decrease in epizootic activity. When natural foci of infection are investigated during periods between epizootics, no antibodies to *Y. pestis* are detected in the animals, and the plague microbe is not detected by bacteriological and biological methods. Morbidity in humans is noted, as a rule, when epizootics become acute, and is a consequence of bites of fleas, direct contact with infected animal tissues, the consumption of insufficiently cooked meat products, or the inhalation of

aerosolized respiratory excreta of animals or patients with the pneumonic form of infection (Anisimov, 2002a, b; Anisimov *et al.*, 2004; Aparin & Golubinskii, 1989; Brubaker, 1991; Butler, 1983; Dennis *et al.*, 1999; Domaradskii, 1993, 1998; Gage & Kosoy, 2005; Hinnebusch, 2003; Inglesby *et al.*, 2000; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Perry & Fetherston, 1997; Pollitzer, 1954).

Plague remains a serious problem for international public health. Small outbreaks of plague continue to occur throughout the world, and at least 2000 cases of plague are reported annually (Gage & Kosoy, 2005). Plague has recently been recognized as a reemerging disease, and *Y. pestis*, if used by the aerosol route of exposure as a bioterrorism agent, could cause mass casualties (Gage & Kosoy, 2005; Inglesby *et al.*, 2000). According to World Health Organization (WHO) data, if 50 kg of the plague pathogen was released as an aerosol over a city with a population of 5 million, 150 000 people might fall ill with pneumonic plague, 36 000 of whom would die (WHO, 1970).

Immunization against plague is one of the major approaches being pursued to deal with potential infection. Commercially available human plague vaccines are based on either a live, attenuated strain or a killed, whole-cell preparation. The live, attenuated vaccine is produced in

Abbreviations: DIC, diffuse intravascular coagulation; WHO, World Health Organization.

the former Soviet Union, and is based on *Y. pestis* strain EV, line NIEG. This strain is attenuated due to deletion of the 102 kb *pgm* locus that includes the *hms* locus responsible for the ability to store haemin, and a cluster of genes needed for production of the siderophore-based yersiniabactin biosynthetic/transport systems. The parental wild-type strain was isolated in Madagascar. The second vaccine is a formalin-fixed virulent *Y. pestis* strain 195/P (originally isolated in India) that was developed in the USA, although at present it is manufactured only in Australia. Both these types of vaccines have unacceptable side effects when used (Meyer, 1970; Naumov *et al.*, 1992). A number of subunit-based vaccines are currently under development and/or at different stages of preclinical/clinical trials. These offer the advantage of employing a defined antigen that possesses the ability to elicit high-level protection, and they are also less reactogenic than the currently used whole-cell vaccines (Anisimov *et al.*, 2004; Williamson, 2001; Williamson *et al.*, 2005). The main limitation of the prevention of plague by vaccination is that protection is delayed for at least 1 week after immunization, and this time may be crucial with respect to the lethal outcome of the disease (Butler, 1983; Dennis *et al.*, 1999; Domaradskii, 1993, 1998; Inglesby *et al.*, 2000; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Perry & Fetherston, 1997; Pollitzer, 1954; Rudnev, 1940). As a consequence, antibiotics are employed for the early initiation of prophylaxis and therapy of plague.

The overwhelming majority of *Y. pestis* natural isolates are susceptible *in vitro* and *in vivo* to antimicrobials such as tetracycline, doxycycline, ciprofloxacin, streptomycin, gentamicin, chloramphenicol, fluoroquinolones, sulphonamides, and trimethoprim-sulfamethoxazole (Domaradskii, 1993; Frean *et al.*, 2003; Inglesby *et al.*, 2000). The infrequent recovery of natural drug-resistant isolates of *Y. pestis* has been explained by the relative rarity of cases of human plague at the present time and by the acute nature of the disease (Domaradskii, 1993). However, a 'natural' strain with resistance to multiple antibiotics, including all of those recommended for plague prophylaxis and treatment, was isolated in 1995 in the Ambalavao district of Madagascar from a 16-year-old boy. The *Y. pestis* strain 17/95 was resistant to ampicillin, chloramphenicol, kanamycin, streptomycin, spectinomycin, sulfonamides, tetracycline and minocycline. The resistance genes were carried by a plasmid that could conjugate with high frequency to other *Y. pestis* strains *in vitro* (Galimand *et al.*, 1997) or in fleas (Hinnebusch *et al.*, 2002). The possibility of the rise of such multidrug-resistant strains in the natural environment, the ease of generation of such strains under laboratory conditions (Hinnebusch *et al.*, 2002; Hurtle *et al.*, 2003), the potential use of such strains for bioterrorist attack, together with the rapidity and high lethality of the disease (Butler, 1983; Dennis *et al.*, 1999; Domaradskii, 1993, 1998; Inglesby *et al.*, 2000; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Perry & Fetherston, 1997; Pollitzer, 1954; Rudnev, 1940), are evidence of the necessity for a search for novel antimicrobial alternatives to antibiotics.

Here, we will briefly review the existing methods, as well as promising current strategies under investigation for the treatment of plague.

Pathogenesis and virulence factors

For constant circulation in natural foci, the plague pathogen must penetrate into the host organism, counteract the protective bactericidal systems of the rodent, and reproduce to ensure bacteraemia, essential for further transmission of the infection by fleas to a new host. In the case of a human pneumonic plague outbreak, the bacteria must also overcome host innate immunity to develop pneumonia with abundant exhalation of *Y. pestis*, causing plague pneumonia in naïve persons. Each of these stages in the cyclic existence of *Y. pestis* is supported by numerous factors of the plague pathogen, including pathogenicity factors and housekeeping genes (Table 1), which may exert an influence, jointly or individually, upon various stages of the infectious process or transmission. The removal of any one of these components may or may not render the organism avirulent. However, only in aggregate do these factors ensure survival of *Y. pestis* in the host organism, no matter how significant or insignificant their individual effect might be (Anisimov *et al.*, 2004; Brubaker, 1991; Perry & Fetherston, 1997).

The main pathogenicity factor of *Yersinia* resides in the complex traits encoded by the plasmid pCad (also termed pCD, pVW, pYV or pLcr), the presence of which in the cell is essential for the manifestation of virulence (Portnoy & Falkow, 1981). It is considered as the Yop virulon: the system that permits extracellular bacteria to disarm the cells involved in the host immune response, disrupt their binding, and induce their apoptosis by injection of bacterial effector proteins. This system consists of the Yop proteins and the apparatus of their type III secretion, called Ysc. The Ysc apparatus consists of 25 proteins, while most of the Yops can be divided into two groups according to their functions. Some of them are intracellular effectors (YopE, YopH, YpkA/YopO, YopP/YopJ, YopM, YopT), whereas others (YopB, YopD, LcrV) form the translocation apparatus, which is unfolded on the surface of the bacterium to deliver the effectors into eukaryotic cells through the plasma membrane. The secretion of Yops proteins is triggered by contact with eukaryotic cells. In the absence of attachment, yersiniae do not release Yops from the bacterial cell. The adhesion mechanism is not yet known, but it seems that close contact with eukaryotic membrane lipids alone might be enough to trigger secretion. The secretion channel may be blocked at different levels by proteins of the virulon, including YopN, YteA and LcrG, which cover the bacterial secretory channel, presumably in the form of a trap door. The precise functioning of the system also requires several chaperones, called Syc proteins, that assist Yops to be secreted by the injectisome. Each of these chaperones serves only one Yop, and they do not leave the bacterial cytosol. Transcription of the genes is controlled by the temperature and activity of the secretion apparatus (Cornelis, 2002; Viboud & Bliska, 2005).

Table 1. Selected factors ensuring survival of *Y. pestis* in the host organism

Compiled from Anisimov, 2002a, b, Anisimov *et al.*, 2004; Brubaker, 1991; Oyston *et al.*, 2000; Perry & Fetherston, 1997; Robinson *et al.*, 2005; Sing *et al.*, 2002; Sodhi *et al.*, 2004. ND, Not determined.

Bacterial factor	Function or activity	Reported decrease of virulence in knock-outs (by order of magnitude)		
		Dramatic (>)	Moderate (4–2)	Low (2–0)
Pathogenicity factors				
Low-calcium response (LCR) stimulon [bacterial virulence-associated type III secretion (T3S) system; <i>Yersinia</i> outer-membrane proteins (Yop) virulon] including:	System permitting extracellular yersiniae to counteract the non-specific immune response by an impairment of phagocytic and signalling activity of macrophages and induction of apoptosis of phagocytic cells (delivery of toxic bacterial proteins, Yops, from extracellularly located bacteria into the eukaryotic cell cytosol)	+	–	–
LcrV	Yop translocator; inhibition of chemotaxis of neutrophils; suppression of the synthesis of γ -interferon and tumour necrosis factor α : cytokines necessary for non-specific activation of professional phagocytes and the formation of productive granulomas, by stimulation of interleukin (IL)-10 production; a repressor of the cytokines indicated above; inhibition of LPS-induced production of IL-1 β in macrophages; competition with LPS for toll-like receptor 2 binding	+	–	–
YopD	Yop translocator	ND	ND	ND
YopE	Antiphagocytic; actin depolymerization; inactivation of Rho proteins	+	–	–
YopH	Antiphagocytic; protein tyrosine phosphatase; induction of apoptosis; inhibition of lymphocyte proliferation	+	–	–
YopM	Disruption of thrombin interaction with thrombocytes and hindering of their aggregation, essential for the formation of a blood clot	+	–	–
YopP/YopJ	Down-regulation of the inflammatory response of macrophages, epithelial and endothelial cells by blocking the mitogen-activated protein kinase and nuclear factor- κ B pathways	–	–	+
YopT	Antiphagocytic; inactivation of Rho proteins; depolymerization of actin stress fibres	ND	ND	ND
YpkA/YopO	Antiphagocytic; inactivation of Rho proteins; autophosphorylating serine/threonine kinase	ND	ND	ND
YscF	Formation of the external needle of the T3S system; participation in virulence protein secretion, in translocation of virulence proteins across eukaryotic membranes and in the cell contact- and calcium-dependent regulation of T3S	ND	ND	ND

Table 1. cont.

Bacterial factor	Function or activity	Reported decrease of virulence in knock-outs (by order of magnitude)		
		Dramatic (>)	Moderate (4–2)	Low (2–0)
Plasminogen activator (Pla)	Spreading factor that promotes generalization of infection, possesses proteolytic properties and determines the fibrinolytic (37 °C) and plasmocoagulase (28 °C) activities of the plague pathogen; post-translational degradation of Yop proteins; adhesive and invasive activity	+	+	+*
pH 6 antigen (PsaA)	Antiphagocytic; adhesive activity	+	+	–
Capsular antigen fraction I (F1; Caf1)	Antiphagocytic; adhesive activity	+	+	+
Ail	Adhesive activity; resistance to bactericidal action of normal sera	–	–	–
<i>Yersinia</i> ‘murine’ toxin (Ymt)	Development of toxic shock (lethal effect on mice and rats); reinforcement of endotoxic shock in mammals	–	–	+
LPS	Development of endotoxic shock; resistance to the action of bactericidal cationic peptides and normal sera; adhesive activity	+	–	–
Factors responsible for nutrient requirements and housekeeping genes				
Yersiniabactin transport and biosynthesis	Siderophore-dependent iron-transport system	+	–	–
Haemin storage (Hms)	Adsorption of haemin on microbial cell surface	–	–	+
Purine biosynthetic enzymes	<i>De novo</i> synthesis of purines	+	–	–
Dam	DNA adenine methylation	–	+	–
Two-component regulatory system PhoPQ	Activation of resistance to innate immune defences	–	–	+

*More than one + indicates the existence of conflicting data reported by different researchers.

Infective doses of 10 bacteria or less are sufficient to cause lethal infection in naïve rodents and primates via the intradermal, subcutaneous and intravenous routes (Anisimov *et al.*, 2004; Brubaker, 1991; Perry & Fetherston, 1997). LD₅₀ values in the case of the respiratory and oral routes of infection increase to 10²–10⁴ c.f.u. (Anisimov, 2002a; Anisimov *et al.*, 2004; Ehrenkranz & Meyer, 1955) and 10⁵–10⁹ c.f.u. (Anisimov, 2002a), respectively.

Y. pestis is primarily a rodent pathogen, and is usually transmitted to another mammalian host by fleas, following the death of the rodent. After being introduced into the host, the bacterium is initially susceptible to phagocytosis and killing by neutrophils (Cavanaugh & Randall, 1959) and CD11c⁺ cells (Bosio *et al.*, 2005), but it may survive and multiply within macrophages. Released bacteria are resistant to capture by neutrophils and begin an unchecked

propagation (Cavanaugh & Randall, 1959). The bacteria then spread from the initial flea-bite site to the lymph nodes, where local replication causes a suppurative lymphadenitis (bubo). If the lymphatic system becomes overwhelmed, septicaemia results, with *Y. pestis* spreading to other organs. The LPS of *Y. pestis* causes the development of endotoxic shock peculiar to other Gram-negative infections (Butler, 1983; Dmitrovskii, 1994; Van Amersfoort *et al.*, 2003). After the development of highly contagious plague pneumonia, the disease spreads in the air in droplets (Lien-Teh, 1926; Lien-Teh *et al.*, 1936; Rudnev, 1940).

Clinical manifestations

There are three main primary forms of human plague, bubonic, septicaemic and pneumonic. Complications such as secondary septicaemic plague, secondary pneumonic

plague, plague meningitis, plague endophthalmitis and multiple lymph node involvement result from bacteraemic dissemination of the pathogen (Butler, 1983; Dennis *et al.*, 1999; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940). Septicaemia may be followed by digital gangrene (Kuberski *et al.*, 2003).

Irrespective of clinical form, the mean incubation period is 3–6 days, but may decrease to 1–2 days in patients with primary septicaemic or pneumonic plague, and increase in vaccinated people. All of the clinical forms may be characterized by sudden onset of illness, malaise, chills and temperature increase up to 39–40 °C, headache, myalgia, insomnia, indistinct speech, wobbly walk, and sometimes vomiting. In the case of serious illness, patients are delirious, and violent and aggressive. Attempts to make an escape from a patient care institution may be considered as a sign of developing meningitis. In extremely severe cases, a profound prostration and cyanosis can be observed.

Fatality for untreated bubonic plague varies from 40 to 60 %, while untreated septicaemic and pneumonic forms of the disease are always lethal (Albizo & Surgalla, 1970; Butler, 1983; Dennis *et al.*, 1999; Dmitrovskii, 1994; Kool, 2005; Krishna & Chitkara, 2003; Lien-Teh, 1926; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940).

Bubonic plague

Bubonic plague results from a flea bite or direct contact of an open skin lesion with plague-infected material. Sometimes, a vesicle, pustule or ulcer develops at the inoculation site. *Y. pestis* spreads via the lymphatic vessels to the regional lymph nodes, causing inflammation and swelling in one or several nodes: buboes ('bubo' is derived from the Greek 'boubon', groin). Buboes are usually no greater than 5 cm in diameter, extremely tender, erythematous, and surrounded by a boggy haemorrhagic area. Buboes typically arise in the inguinal and femoral regions, but also occur in other regional lymph node sites, including popliteal, axillary, supraclavicular, cervical, post-auricular, pharyngeal and other sites. Deeper nodes (such as intrabdominal or intrathoracic nodes) may also be involved through the lymphatic or haematogenous spread of *Y. pestis*. Initial symptoms include malaise, high fever, and one or more painful lymph nodes. Pulse rate is increased to 110–140 min⁻¹. Blood pressure is low, usually about 100/60 mm Hg, due to extreme vasodilatation. Circulatory collapse, haemorrhage and peripheral thrombosis are the terminal events (Butler, 1983; Dennis *et al.*, 1999; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940).

Septicaemic plague

Septicaemic plague may occur primarily as a result of infection with massive doses of the pathogen, or when the bacillus is deposited in the vasculature, bypassing the lymphatics. In this case, bacteria proliferate in the body without producing a bubo. Secondary septicaemic plague is

a complication of haematogenous dissemination of bubonic plague. The presenting signs and symptoms of primary septicaemic plague are essentially the same as those of any Gram-negative septicaemia: fever, chills, nausea, vomiting and diarrhoea. Later, purpura, disseminated intravascular coagulation, and acral cyanosis and necrosis may be seen. Other symptoms include abdominal pain and digital gangrene (Albizo & Surgalla, 1970; Butler, 1983; Dennis *et al.*, 1999; Dmitrovskii, 1994; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940; Van Amersfoort *et al.*, 2003).

Pneumonic plague

Pneumonic plague is rare, but is the most dangerous and fatal form of the disease, and is spread via respiratory droplets. It can develop as a secondary complication of septicaemic plague or result from the inhalation of infectious respiratory excreta of humans or animals with the pneumonic form of infection. The onset of pneumonic plague is acute and fulminant, with high fever, chills, headache, malaise, myalgia and productive cough (with sputum that may be clear, bloody or purulent) within 24 h of the onset of symptoms. Buboes on the neck are rare, but are a symptom of pneumonic plague. Nausea, diarrhoea, vomiting and abdominal pain may also accompany the disease. The pneumonia progresses rapidly, resulting in dyspnoea, stridor and cyanosis. The disease rapidly engulfs the lungs and haemorrhages develop, filling them with fluid (a haemorrhagic pneumonia). The terminal events are respiratory failure, circulatory collapse, and bleeding diathesis (Albizo & Surgalla, 1970; Butler, 1983; Dennis *et al.*, 1999; Dmitrovskii, 1994; Kool, 2005; Krishna & Chitkara, 2003; Lien-Teh, 1926; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940).

Methods of plague treatment

Until the nineteenth century, the treatment of plague was based on mysticism and superstition. Such 'remedies' as magic and talismans, mixtures of bird and animal blood, tablets made from rattlesnake meat, and even material squeezed from fresh horse dung, were widely used (Afanas'ev & Vaks, 1903). Later, methods such as phlebotomy, emetics, purgatives and diaphoretics were also applied to plague treatment (Rudnev, 1940). The isolation of the plague pathogen, *Y. pestis*, in 1894 (Yersin, 1894) made possible scientific approaches to the cure of plague-infected patients. The local application of antiseptics such as iodine, mercuric chloride, carbolic acid or quinine, together with the incision or even searing of buboes, were promising in some cases, as were attempts to surmount severe systemic disease by the use of the same remedies, specific bacteriophages or animal hyperimmune sera (Lien-Teh *et al.*, 1936; Rudnev, 1940); however, real success in plague therapy was observed when sulfanilamides (Carman, 1938) and then streptomycin (Hornibrook, 1946) became available.

Antibiotic prophylaxis and therapy

Currently, antibiotics are the cornerstone of plague treatment. The antibiotic prophylaxis and therapy for *Y. pestis* infection, as suggested by the WHO Expert Committee on Plague (1970), focuses on the use of tetracycline, streptomycin, and chloramphenicol for eradication of the organism. More recently, the US Working Group on Civilian Biodefence has added gentamicin, doxycycline and ciprofloxacin to this list (Inglesby *et al.*, 2000). Chloramphenicol is optional for the treatment of plague meningitis (Becker *et al.*, 1987). Aminoglycosides (streptomycin, kanamycin, tobramycin, gentamicin and amikacin) and cephalosporins (ceftriaxone and ceftazidim) are recommended for the treatment of plague caused by F1-negative *Y. pestis* strains that are resistant to doxycycline, ampicillin and cefoperazone *in vivo*. Studies in mice suggest that an increase in the daily doses of less-efficient drugs such as cefotaxime, cefoperazone, sulbactam/ampicillin, aztreonam, ciprofloxacin and rifampicin, along with prolongation of the treatment course for up to 7 days, make it possible to increase the protective effects to 80–100% (Anisimov *et al.*, 2004; Ryzhko *et al.*, 1998). Furthermore, studies using ciprofloxacin, doxycycline and the newer fluoroquinolones gatifloxacin and moxifloxacin have shown an increase in the survival of mice presenting with pneumonic plague (Byrne *et al.*, 1998; Russell *et al.*, 1998; Steward *et al.*, 2004).

It is recommended that antibiotic therapy be started as early as possible. A delay in antibiotic therapy will result in an increase in bacterial biomass, and in a more harmful subsequent inflammatory response caused by endotoxin release induced by both lytic and non-lytic antimicrobials (Nau & Eiffert, 2002). During the first few hours of antibiotic treatment, patients should be monitored closely, because shock may develop after the start of antibiotic application due to bacteriolysis, with the subsequent release of large amounts of endotoxin (Jacobs *et al.*, 1990). Those in contact with pneumonic plague patients and persons who have been exposed to aerosols should receive doxycycline, ciprofloxacin or chloramphenicol as post-exposure prophylaxis (Inglesby *et al.*, 2000). The recent increase in multidrug-resistant strains (Galimand *et al.*, 1997; Wong *et al.*, 2000) has led to renewed efforts to find alternatives to antibiotics.

Immunotherapy

Serum therapy. A. Yersin and others were the first to use the serum of vaccinated rabbits to cure infected animals (Yersin *et al.*, 1895). In 1896, Yersin was able to cure several patients in Asia with a horse serum (Yersin, 1897). However, the experiences of other researchers with the use of plague serum were conflicting. The horse, mule or bull sera were produced in different laboratories with the use of different immunization schemes involving killed or live bacteria of different strains. In the course of treatment, patients were injected subcutaneously or intravenously with up to 1000 ml immune serum (200–500 ml for one subcutaneous or intramuscular injection, and

50–100 ml for one intravenous injection). In the case of bubonic plague, the reported death rate in treated (13%) and untreated (64%) patients differed significantly. In patients with septicaemic or pneumonic plague, treatment did not cause a detectable decrease in mortality. The sooner treatment was started the better the result. Even in patients that succumbed to infection, an up to twofold increase in time to death was usually reported. The dangers of serum sickness and anaphylactic shock frequently induced by serum therapy were believed to be less significant than that of septic shock (Lien-Teh *et al.*, 1936; Pollitzer, 1954; Rudnev, 1940). In 1970, the WHO Expert Committee on Plague recommended the continuation of further development of plague antitoxic sera that might be used for the treatment of plague patients with severe toxicosis (WHO, 1970).

Antibody therapy. Beginning in the 1960s, separate antibody preparations were introduced into clinical practice for preventing and treating infectious diseases. In contrast to older remedies based on animal sera, these specific immunoglobulins contained antibodies derived from immunized human donors, and so had minimal side effects. Although human-specific globulins lack the toxicities associated with animal sera, they have several limitations, such as high cost, low availability, and the potential to transmit infectious disease (Buchwald & Pirofski, 2003; Casadevall, 2002; 2005; Keller & Stiehm, 2000).

Hybridoma technology, introduced in 1975 (Kohler & Milstein, 1975), provides a method for the unlimited production of homogeneous mAbs. The construction of mouse-human chimeric or humanized mAbs (Morrison, 1992), completely human mAbs (Kang *et al.*, 1991) from hybridomas or combinatorial libraries, and recombinant human polyclonal antibodies (Bregenholt & Haurum, 2004), makes it possible to reduce the immunogenicity of rodent mAbs in humans. These approaches provide the possibility of raising relevant antibodies against protective antigens only, and even against protective epitopes of protective antigens.

In the case of *Y. pestis*, several antigens have been shown to be able to produce protective immunity. Among them are F1 capsular antigen (Meyer *et al.*, 1974; Simpson *et al.*, 1990), LcrV or simply V antigen (Leary *et al.*, 1995; Motin *et al.*, 1994; Une & Brubaker, 1984), YopD (Andrews *et al.*, 1999), and type III secretion system needle complex protein, YscF (Matson *et al.*, 2005). Immunodominant epitopes have been found for the F1 (Sabhnani & Rao, 2000; Sabhnani *et al.*, 2003; Zav'yalov *et al.*, 1995) and V (Hill *et al.*, 1997) antigens. Passive administration of antibodies against target antigens protects macrophages from *Y. pestis*-induced cell death, promotes phagocytosis (Cowan *et al.*, 2005; Philipovskiy *et al.*, 2005; Weeks *et al.*, 2002), and protects animals against both bubonic and pneumonic plague (Anderson *et al.*, 1997; Friedlander *et al.*, 1995; Green *et al.*, 1999; Hill *et al.*, 2006; Motin *et al.*, 1994; Roggenkamp

et al., 1997; Une & Brubaker, 1984; Williamson *et al.*, 2005). Importantly, therapy based on a single antibody against a single antigen or epitope will be ineffective in the case of infection with a virulent strain lacking the antigen or expressing a different serological variant of the antigen (Anisimov *et al.*, 2004; Friedlander *et al.*, 1995; Roggenkamp *et al.*, 1997).

Non-pathogen-specific immunomodulatory therapy. *Y. pestis* is known to efficiently overcome the innate immune system of many mammals. However, it has been shown that neutrophils (Cavanaugh & Randall, 1959) and CD11c⁺ cells (Bosio *et al.*, 2005), which represent the initial line of host defence against invading pathogens, play an important role in suppressing the initial replication and dissemination of inhaled *Y. pestis*. Recent studies (Liles, 2001) conducted *in vitro* and *in vivo* have shown that granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor and interferon- γ can augment the functional antimicrobial activities of neutrophils. Studies conducted in animal models have shown the potential use of each of these cytokines for the treatment of bacterial infections. We can speculate that such adjunctive treatment may be useful for plague therapy.

Y. pestis mediates septic shock, which contributes significantly to host death (Butler, 1983; Dmitrovskii, 1994). Sepsis and the systemic inflammatory response syndrome are accompanied by the inability to regulate the inflammatory response (Van Amersfoort *et al.*, 2003). The most potent class of cholesterol-lowering drugs, statins, has been shown to also possess cholesterol-independent effects, including diverse immunomodulatory and anti-inflammatory properties (Almog, 2003). Recent studies have demonstrated that simvastatin (Merx *et al.*, 2004) and cerivastatin (Ando *et al.*, 2000) pretreatment profoundly improves survival in a murine model of sepsis. Another retrospective study has shown that statin therapy reduces both overall and attributable mortality in patients with bacteraemia (Liappis *et al.*, 2001). The lipid profiles of the statin-pretreated patients were considerably higher than those of the no-statin group, and it has been suggested that the medicinal efficacy is a result of the attenuation of the intensity of the inflammatory response due to binding of endotoxins by increased amounts of lipids (Almog *et al.*, 2004).

Several other immune response modifiers that target different stages of innate immunity and might be useful for plague therapy have recently been reviewed (Amli-Lefond *et al.*, 2005; Masihi, 2000), but only one, glutoxim, a sulfur-containing hexapeptide with an immunomodulatory effect on lymphocytes (Fimiani *et al.*, 2002), has been used in an animal model of plague (Zhemchugov, 2004). When injected into mice (12.5 mg per animal) 6 h before challenge with 10 LD₅₀ of the virulent *Y. pestis* strain 231, it protected half of the animals from death. Glutoxim has also been shown to protect mice from tularaemia and

meliodosis. However, the data thus far are inconclusive, as they were obtained in isolated experiments that employed a minimal number of laboratory animals.

Phage therapy

Bacteriophages seem to be good candidates for antibacterial therapy: they often possess high species specificity, they are non-toxic to eukaryotes, and they kill the target bacteria that they infect and within which they multiply. After the discovery of bacteriophages by Twort in 1915, and independently by d'Herelle in 1917, a number of researchers and physicians were overenthusiastic in the use of bacteriophages for the therapy of bacterial disease (Skurnik & Strauch, 2006; Sulakvelidze, 2005; Summers, 1999, 2001). With respect to plague, in 1925, d'Herelle used a highly virulent anti-plague phage that had been isolated in 1920 in Indo-China from rat faeces to treat four cases of bubonic plague as the first bacteriophage therapy (d'Herelle, 1925). All the patients had laboratory-diagnosed bubonic plague with very serious clinical signs. d'Herelle treated all four with anti-plague phage preparations by single/double direct injection of phage (0.5–1 ml) into the buboes. Within several hours of injection, the patients felt better, a 2 °C average fall in temperature was recorded, and severe pain in the buboes decreased. All four patients recovered in what was considered a remarkable fashion. On the basis of this work, a number of attempts to confirm the efficacy of the phage therapy of plague both in animal models and in clinical trials were performed (d'Herelle, 1925; Flu, 1929; Fonquernie, 1932; Lien-Teh *et al.*, 1936; Naidu & Avari, 1932; Rudnev 1940). However, a poor understanding of the mechanisms of phage–bacterial interactions, including lysogeny and phage DNA restriction, and poorly designed and executed experiments and clinical trials, together with the use of undefined phages in the form of non-purified phage preparations, led to conflicting results (Skurnik & Strauch, 2006; Sulakvelidze, 2005; Summers, 1999, 2001).

Bacteriophages have received renewed attention as possible agents against bacterial pathogens. Evidence from several recent trials designed and executed in accordance with good laboratory practice (GLP) regulations indicates that phage therapy can be effective, although the efficacy of the phage treatment depends greatly on the route (oral, intramuscular, aerosol spray, etc.) and/or frequency of phage application (Skurnik & Strauch, 2006; Sulakvelidze, 2005; Summers, 1999, 2001). Numerous obstacles to the use of phage as antimicrobials for clinical practice remain. Among them are the issue of phage resistance, and the possibility of phage-mediated transfer of undesirable genetic material to bacterial hosts. Therapy based on the simultaneous use of several phages with known genetic sequences and targeting different bacterial receptors can help in overcoming these obstacles. Currently, the genomes of several *Y. pestis*-specific phages have been sequenced (Filippov *et al.*, 2005; Garcia *et al.*, 2003), and they could be candidates for the revival of plague phage therapy.

A phage-encoded peptidoglycan-degrading activity is responsible for the cell-wall-hydrolysing action of bacteriophages. At least four types of these enzymes, endolysins, are responsible for this activity in different phages (Young, 1992). Taking into account that if a sufficiently high phage dose is given, phages are able to control infection through a non-proliferative lytic mechanism (Berchieri *et al.*, 1991; Goode *et al.*, 2003), the therapeutic use of endolysins in a controlled fashion seems advantageous in comparison with the uncontrollable increase of the live active agent.

Bacteriocin therapy

Bacteriocins, bacterially produced antimicrobial peptides, range from the well-studied, narrow spectrum, high-molecular-mass colicins produced by *Escherichia coli* and the short polypeptide lantibiotics of lactic acid bacteria to the relatively unknown halocins produced almost universally by halobacteria. Purified bacteriocins could be used for the reduction or elimination of certain pathogens (Braude & Siemieniowski, 1965; Riley & Wertz, 2002). It has been shown in assays for potency that purified colicins V and K have similar inhibitory activities on a per weight basis to those of the therapeutic antibiotics kanamycin, streptomycin and oxytetracycline (McGeachie, 1970). Bacteriocins nisin A (produced by *Lactococcus lactis*) and mutacin B-Ny266 (produced by *Streptococcus mutans*) are as active as vancomycin and oxacillin against most strains tested (*Bacillus*, *Enterococcus*, *Lactococcus*, *Listeria*, *Mycobacterium*, *Pediococcus*, *Staphylococcus*, *Bordetella*, *Clostridium*, *Peptostreptococcus*, *Propionibacterium*, *Streptococcus* and *Micrococcus*). Furthermore, mutacin B-Ny266 remains active against strains that are resistant to nisin A, oxacillin and vancomycin (Mota-Meira *et al.*, 2000).

Intraperitoneal injections of mutacin B-Ny266 have been used for the treatment of mice infected with *Staphylococcus aureus*. While there was 100% mortality in the control group of mice, no mortality was observed in the mice injected with vancomycin or mutacin B-Ny266 (Mota-Meira *et al.*, 2005). Lacticin 3147, a broad-spectrum bacteriocin produced by the food-grade organism *L. lactis*, reduced the incidence of mastitis after experimental challenge with *Streptococcus dysgalactiae* in non-lactating dairy cows (Ryan *et al.*, 1999). Local injections of staphylococcal A-1262a were used to treat 50 patients with a variety of staphylococcal lesions. Complete recovery was observed in 42 of the patients (Lachowicz, 1965). The streptococcal bacteriocin tomicide was used for protection of white mice from staphylococcal infection. Tomicide, administered in the maximum dose admissible for mice, ensured the protection of up to two-thirds of the total number of mice. A single oral administration of the preparation immediately after infection protected one-third of the surviving mice from local staphylococcal infection (Blinkova *et al.*, 2003). The pronounced prophylactic effect of tomicide, manifested by a reliable decrease of group A streptococcal carrier state, as well as by a decrease in morbidity in respiratory streptococcal infection among

children in a test group in comparison with controls, has been reported (Briko & Zhuravlev, 2004). Enterocolitacin, a phage-tail-like bacteriocin, has been administered as an antimicrobial compound by the oral route for the treatment of BALB/c mice infected with *Yersinia enterocolitica*, the nearest relative of *Y. pestis*. The increase in the number of *Y. enterocolitica* c.f.u. in animals was retarded at time points shortly after the application of enterocolitacin, indicating that the bacteriocin was effective during the early phase of infection (Damasko *et al.*, 2005). A purified class IIa bacteriocin, secreted by *Paenibacillus polymyxa* NRRL-B-30509, has been incorporated into chicken feed, and dramatically reduced both intestinal levels and the frequency of chicken colonization by *Campylobacter jejuni* (Stern *et al.*, 2005). The same bacteriocin has been shown to inhibit the growth of *Y. pestis* *in vitro* (E. A. Svetoch and B. V. Eruslanov, personal communication) in doses similar to those recommended by the WHO Expert Committee on Plague (WHO, 1970) for antibiotics. The above studies suggest that there is a potential therapeutic effect associated with bacteriocins. When contemplating the clinical use of bacteriocins, one important consideration is their possible pathological effects. In early studies with partially purified bacteriocins (Montgomerie *et al.*, 1973; Tagg & McGiven, 1972; Turnowsky *et al.*, 1973) or strain pairs differing in their expression (Brubaker *et al.*, 1965; Burrows, 1965; Smith, 1974), the data showed their potential toxicity, and also a relationship between the carriage of bacteriocinogenic factors and the virulence of certain strains. However, studies with purified substances have in many cases failed to confirm toxicity (Braude & Siemieniowski, 1965; Tagg *et al.*, 1976). Although intraperitoneal injection of 10 mg mutacin B-Ny266 kg⁻¹ did not apparently affect the health of mice, these results will have to be confirmed with more relevant toxicity tests (Mota-Meira *et al.*, 2005). The investigation of the *in vitro* cytotoxicities of two bacteriocins, gallidermin (*Staphylococcus gallinarum*) and nisin A, in comparison with those of antimicrobial peptides of eukaryotic origin, magainin I, magainin II and melittin, indicated that gallidermin was the least cytotoxic antimicrobial peptide, followed by nisin A, magainin I, magainin II and melittin. Nisin caused haemolysis, but at concentrations which were 1000-fold higher than those required for antimicrobial activity. Gallidermin shows the most promise as a therapeutic agent, with relatively low cytotoxicity and potent antimicrobial activities (Maher & McClean, 2006). In considering the potential toxicity of bacteriocins, the well-known toxicity of antibiotics such as quinolones, doxycycline, streptomycin and gentamicin, and the possible problems associated with massive use of antimicrobial agents for prophylaxis or therapy during a bioterrorist attack, should be noted (Navas, 2002).

Inhibitors of virulence factors

Virulence refers to the ability of an organism to establish an infection and cause disease. Many steps are involved in the infection process, including adherence, invasion and the evasion of host defences (Finlay & Falkow, 1997). Microbial

factors involved in the process of virulence are unique, in that their inhibition, by definition, should interfere with the process of infection rather than with bacterial viability. Inhibitors of such targets would be unlikely to affect host cells, to be cross-resistant to existing therapies, or to induce resistance themselves. Bacterial virulence may therefore offer unique opportunities to inhibit the establishment of infection or alter its course as a method of antimicrobial chemotherapy (Alksne, 2002; Y. M. Lee *et al.*, 2003; Marra, 2004).

A necessary step in the successful colonization and, ultimately, production of disease by microbial pathogens is the ability to adhere to host surfaces (Finlay & Falkow, 1997), in many cases involving oligosaccharides located on the host cell surface and bacterial adhesins. The terminal di- or trisaccharide units of these oligosaccharides may be used to inhibit these interactions, preventing attachment and therefore disease. The validity of this approach has been unequivocally demonstrated in experiments performed in a wide variety of animals, from mice to monkeys, and also in humans (Ofek *et al.*, 2003).

Y. pestis expresses a range of adhesins, including pH 6 (PsaA), F1 (Caf1), Pla and S-layer protein (Anisimov, 2002b; Kienle *et al.*, 1992; Parkhill *et al.*, 2001; Payne *et al.*, 1998). pH 6 antigen binds to several human immunoglobulin G subclasses by acting as a bacterial Fc receptor (Zav'yalov *et al.*, 1996). It is also able to bind to gangliosylceramide, gangliosylceramide and lactosylceramide, and also to attach to hydroxylated galactosylceramide. Recombinant pH 6 antigen, present on the surface of intact *E. coli* cells, possesses the same specificity as the purified antigen, with the exception of binding to non-hydroxylated galactosylceramide. The observed binding patterns indicate that the presence of β 1-linked galactosyl residues in glycosphingolipids is the minimum requirement for binding of the pH 6 antigen. The glycosphingolipids recognized by the pH 6 antigen are common and may be found on a range of host cell types (Payne *et al.*, 1998). In fact, pH 6 antigen preparations are able to agglutinate human, rabbit, guinea pig and murine erythrocytes (Bichowsky-Slomnicki & Ben-Efraim, 1963). Studies that are currently in progress in the Laboratory for Plague Microbiology, Department of Infectious Diseases, State Research Center for Applied Microbiology and Biotechnology, indicate that pH 6 antigen is capable of promoting the adhesion of macrophage-like eukaryotic cells to each other and to plastic surfaces, and the formation of cell monolayers. The high-molecular-weight capsular antigen F1 possesses haemagglutinating activity on account of its ability to bind specifically to D-galactosamine-HCl and glucuronic acid (Anisimov, 2002b). An analysis of the complete genome of *Y. pestis* has revealed the presence of eight more gene clusters similar to the operons *psa* and *caf1*, each of which is potentially capable of promoting the expression of the pilus adhesins (Parkhill *et al.*, 2001). Transfer of the *pla* locus, which encodes the production of the plasminogen activator Pla, to *E. coli* cells imparted adhesive activity to the latter with respect to a number of

eukaryotic cells (Kienle *et al.*, 1992), on account of the ability of Pla to bind to the mammalian extracellular matrix (Lähtenmäki *et al.*, 1998). *Y. pestis* S-layer protein at a concentration of 8–16 $\mu\text{g ml}^{-1}$ agglutinated rabbit erythrocytes; this agglutination was inhibited by 0.1 M sugars: rhamnose (fourfold) and dulcitol (eightfold) (Diatlov & Antonova, 1999). Until recently it was customarily considered that the plague pathogen had lost the ability to synthesize adhesin/invasin Ail on account of the insertion of IS285 (Brubaker, 2004); however, in the genome of the *Y. pestis* virulent strain CO92, this gene is intact (Parkhill *et al.*, 2001). More recently, the *Y. pestis* genome has been shown to possess two homologues to the *Erwinia* HecA adhesin (Rojas *et al.*, 2002). *Y. pestis* lipopolysaccharide is also reported to be an adhesin (Straley, 1993).

Y. pestis, as a species associated with pneumonic infections, adheres more efficiently to an alveolar epithelial A549 cell line than enteric bacteria, including enteric yersiniae. The plague pathogen demonstrates a restricted tropism for oligosaccharides compared with environmental and opportunistic bacteria. It has been shown that the compound with the greatest anti-adhesion activity toward A549 cells is *p*-nitrophenol (Thomas & Brooks, 2004). As an alternative to antibiotics, the inhibition of attachment can be mediated through the use of oligosaccharide receptor mimics. In a more recent report, Thomas & Brooks (2006) have demonstrated that the attachment of *Y. pestis* strain GB to the murine monocyte cell line J774A.1 and a range of human respiratory epithelial cell lines is reduced by 55–65 % after pre-treatment of the cell lines with tunicamycin (an inhibitor of the biosynthesis and processing of *N*-linked oligosaccharides, produced by *Streptomyces lysosuperificus*). This further demonstrates the potential of oligosaccharides as anti-adhesion therapeutics. Other generic attachment inhibitors include polymeric saccharides (dextran and heparin), GalNAc β 1-4Gal, GalNAc β 1-3Gal, Gal β 1-4GlcNAc and Gal β 1-3GlcNAc (Thomas & Brooks, 2004). It is possible that mixtures of such compounds may serve as a novel class of therapeutics for respiratory tract infections, including pneumonic plague.

The Ysc-Yop-‘type III’ weaponry delivering effector proteins into the cytosol of the eukaryotic target cell seems to be the main pathogenicity factor of *Yersinia*. Inhibitors specifically targeting type III secretion are attracting attention (Chen *et al.*, 2003; K. Lee *et al.*, 2003, 2005; Kauppi *et al.*, 2003a,b; Nordfelth *et al.*, 2005; Xie *et al.*, 2004). The majority of them target YopH, protein tyrosine phosphatase (Chen *et al.*, 2003; K. Lee *et al.*, 2003, 2005; Xie *et al.*, 2004) or YopE, a GTPase-activating protein (Kauppi *et al.*, 2003b). Among such inhibitors are compounds that belong to a class of acylated hydrazones of different salicylaldehydes (Nordfelth *et al.*, 2005), monoanionic squaric acids (Xie *et al.*, 2004), peptidic α -ketocarboxylic acids, and sulfonamides (Chen *et al.*, 2003). A eukaryotic cell model that mimics *in vivo* conditions has shown that some of the inhibitors attenuate the pathogen to the advantage of the host cell.

Iron is a necessary nutrient for all pro- and eukaryotic cells, and at physiological pH values, iron salts (Fe^{3+}) form an almost-insoluble ferric hydroxide, $\text{Fe}(\text{OH})_3$. To assimilate the negligible portion of the iron that is still present in the dissolved state, both in the mammalian organism and in micro-organisms, special iron-binding molecules, siderophores, are synthesized. Bacterial siderophores have a critical role in the competition between parasite and host for iron acquisition (Braun, 2001; Finlay & Falkow, 1997). Therefore, the *Y. pestis* siderophore yersiniabactin, as well as its receptor Psn (Perry & Fetherston, 1997), are promising targets for the development of new antimicrobial inhibitors to treat plague. An inhibitor of the domain salicylation enzymes required for siderophore biosynthesis in *Y. pestis*, the aryl acyl adenylate analogue 5'-O-[N-(salicyl)-sulfamoyl] adenosine, has recently been designed, synthesized, and shown to inhibit yersiniabactin biosynthesis and the growth of *Y. pestis* under iron-limiting conditions (Ferrerias *et al.*, 2005). More recently, a set of newly synthesized aryl sulfamoyl adenosine derivatives has also been shown to inhibit yersiniabactin biosynthesis *in vitro* (Miethke *et al.*, 2006).

Other possible targets for inhibiting *Y. pestis* virulence are quorum sensing (Suga & Smith, 2003), the two-component regulatory systems (Oyston *et al.*, 2000; Winfield *et al.*, 2005) that govern virulence, and/or the enzymes for the biosynthesis of the LPS that is believed to determine antimicrobial-cationic-peptide (Anisimov *et al.*, 2005; Bengoechea *et al.*, 1998) and serum resistance (Anisimov *et al.*, 2005; Porat *et al.*, 1995).

Symptomatic treatment

Severe cases of plague are characterized by shock and extensive diffuse intravascular coagulation (DIC), two interrelated processes that have common causal mechanisms and reinforce each other (Hardaway, 1982). The standard treatment of the pathophysiological changes accompanying endotoxic shock and DIC consists of administration of fluid and vasopressors to restore blood pressure and organ blood flow, and oxygenation (Cohen & Glauser, 1991; Naumov & Samoilova, 1992; Rackow & Astiz, 1991; Wheeler & Bernard, 1999).

Very important to the management of shock is early fluid resuscitation and the immediate start of mechanical ventilation (Carcillo *et al.*, 1991). In addition to extensive capillary leakage, severe plague cases are characterized by severe cardiac depression (Albizo & Surgalla, 1970; Butler, 1983; Dennis *et al.*, 1999; Dmitrovskii, 1994; Lien-Teh *et al.*, 1936; Naumov & Samoilova, 1992; Nikolaev, 1972; Pollitzer, 1954; Rudnev, 1940; Van Amersfoort *et al.*, 2003). Consequently, pulmonary congestion may develop early, and this limits the amount of fluid that can be administered. In general, inotropic and vasopressive support is needed from an early stage. Dobutamine is preferred for its beneficial effects on cardiac function and peripheral oxygenation (Vincent *et al.*, 1990; Winslow *et al.*, 1973).

As for the anti-DIC therapy, currently the only undoubtedly successful treatment is anti-shock therapy. Bleeding due to DIC should probably be treated with replacement therapy: platelets for thrombocytopenia, cryoprecipitate for hypofibrinogenaemia and fresh frozen plasma for decreased coagulation factors (Jacobson & Young, 1986). DIC may cause severe distal necrosis (Welty *et al.*, 1985), and if gangrene has completely demarcated then plastic surgery or amputation may be needed (Kuberski *et al.*, 2003). In the case of evolving gangrene, in a patient with good peripheral blood supply and no bleeding diathesis, sympathetic blockade to preserve peripheral feet tissues has been shown to be successful (Kuberski *et al.*, 2003).

Concluding remarks

The treatment dynamics of plague are of critical interest because of the high human mortality rate of the disease, and current threat for use in bioterrorism. Even though antibiotics such as ciprofloxacin, doxycycline and the newer fluoroquinolones have shown an increase in survival in mice presenting with pneumonic plague, there has recently been a worrying increase in multidrug-resistant *Y. pestis* strains. This has necessitated the need to look at alternatives to antibiotics as treatment regimens for plague. This review has highlighted some of the promising non-antibiotic therapeutic strategies that can be explored further. Thus, a novel treatment mechanism of pneumonic plague infection may be via inhibiting adhesion of *Y. pestis* to the respiratory tract. Recent progress in the use of short-chain oligosaccharides as potential anti-adhesion therapeutics, and also a set of newly synthesized aryl sulfamoyl adenosine derivatives as inhibitors of yersiniabactin biosynthesis, make these receptor mimics potential alternatives to antibiotics. Although hopes remain high that these alternative strategies of plague treatment, alone or as part of combination therapies, will provide a valuable new line of defence against multidrug-resistant *Y. pestis* strains, they remain a long way from clinical practice. The alternative therapies reported in this review should be investigated by comprehensive studies of their clinical applications as a form of preparedness against any possible bioterrorist attack involving the use of antibiotic-resistant *Y. pestis*.

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