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REVIEW ARTICLE

# Tropical and exotic infections

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## Introduction

1998 marked the centenary of the foundation of the Liverpool School of Tropical Medicine (LSTM) and it is fitting that this 5th annual LSTM Bayer Symposium was devoted to tropical and exotic infections. It was one of the first of numerous special events held during the year to celebrate the founding of the School, the subsequent history of which has been described in detail by Power [1] and in pictorial format by Miller [2].

Liverpool has always played a leading role in the control of infectious diseases, starting with the appointment of the first Medical Officer of Health, William Henry Duncan, in 1847 [3]. Duncan's major contribution was his recognition that the control of infections such as cholera, tuberculosis and diphtheria required a robust approach to improving the squalid housing conditions that prevailed in Liverpool at the time. Although his methods had considerable success, there still remained the threat of infections imported from elsewhere by the many travellers passing through the thriving port. The end of the nineteenth century heralded a new era of scientific understanding about the pathogenesis and transmission of infections, with a steady stream of newly described bacteria, parasites and their vectors (Table 1), paving the way for control strategies based on ecological principles, and with the potential for vaccine development.

Within this atmosphere of scientific discoveries, at the peak of the extent of the British Empire, it was recognised that tropical medicine training should be introduced into undergraduate medical curricula. Meanwhile, Liverpool shipping firms faced continued economic losses due to the ill health of their employees while abroad or on their return. These and other pressures led the medical staff of the Royal Southern Hospital to discuss the establishment of an institution in Liverpool that would provide a focus for interest in research, management and control of tropical disease. Alfred Lewis Jones, owner of the Elder Dempster Line, took up the challenge of facilitating this proposal politically and financially, and the Committee that governed the 'School of Tropical Diseases and Medical Parasitology' was established in 1898. The first laboratory and the new ward in the Royal Southern Hospital were officially opened early in 1899, about 6 months before the equivalent School of Hygiene and Tropical Medicine in London [5], and formal taught courses in hygiene and in tropical medicine started the same year.

The new Tropical Ward was usually full, the majority of patients working in the shipping trade, but the absolute number of patients was not huge. There were 1801 admissions between 1899 and 1910, of which 74% were for malaria (Fig. 1), which often required prolonged lengths of stay. These patients provided clinical material for teaching, similar to arrangements that continue to this day. Apart from training, much of the work of the School staff was driven by involvement in the 32 overseas expeditions sent from the School before the First World War, as well as contributions of staff to several other expeditions.

In the century since the School was founded, the global threat from infection has continued to expand rather than recede. Although there have been a few spectacular success stories, such as the eradication of smallpox, many diseases amenable to control by vaccination are still prevalent due to failure of the infrastructures necessary to deliver supplies to the populations at risk. Initial success in the control of malaria was followed by resurgence, aided by the emergence of insecticide-resistant vectors and drug-resistant parasites. Changes in living conditions, including urbanisation, encroachment on forested areas and changes in animal husbandry all encourage the

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Table 1. Selected pathogen 'discoveries' in the period leading up to 1898 (from ref. [4])

| Disease | Place | Person | Year |
|---|---|---|---|
| Cholera | India | Koch | 1884 |
| Typhoid | Switzerland | Eberth | 1880 |
| Plague | Hong Kong | Yersin | 1894 |
| Brucellosis (melitensis) | Malta | Bruce | 1887 |
| Brucellosis (abortus) | Denmark | Bang | 1897 |
| Cutaneous leishmaniasis | India | Cunningham | 1885 |
| Cutaneous leishmaniasis | Tashkent | Borovsky | 1898 |
| Malaria (falciparum) | Algeria | Laveran | 1880 |
| Malaria (malariae) | Italy | Marchiafava and Celli | 1884 |
| Malaria (mosquito vector) | India | Ross | 1897 |
| Paragonimiasis | China | Manson | 1880 |
| Paragonimiasis | Japan | von Baelz | 1880 |

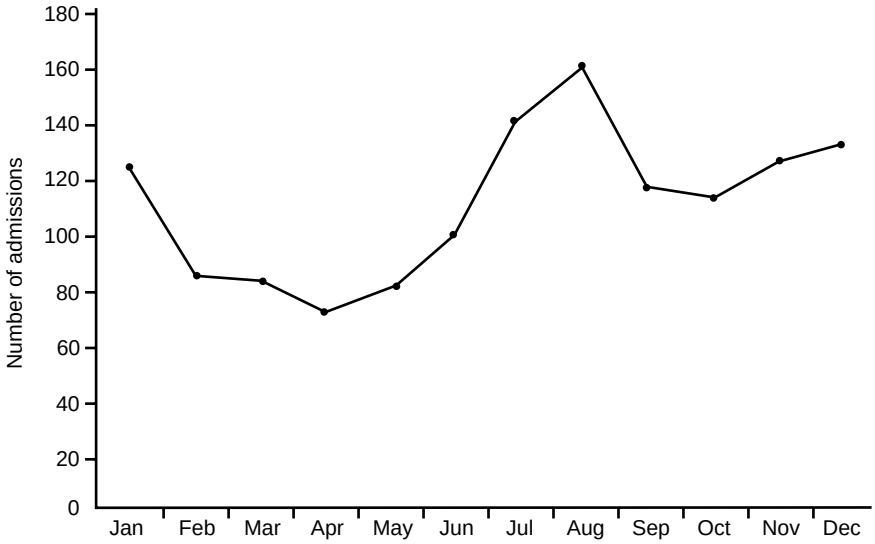


Fig. 1. Malaria admissions to the Tropical Ward, Royal Southern Hospital, Liverpool 1899–1910 (reproduced from ref. 1, with permission).

emergence of new pathogens. Natural disasters, war and other pressures causing movements of displaced persons also contribute to the emergence of pathogens in new areas and re-emergence of old ones. The exponential increase in both volume and distance of air travel means that Britons are at increasing risk of encountering and importing infections from abroad. The clinical discipline of travel medicine is slowly evolving to cater for this important group, aided by the phenomenal recent growth in electronic information sources on emerging infections and travellers' health (Table 2).

It is clear that the role of the School of Tropical Medicine remains as vital as ever, continuing its primary mission to improve the health of people in the tropics through research and training, and providing a focus of diagnostic, clinical and preventive expertise for those potentially exposed to infection while travelling. The papers presented at this Symposium provide a glimpse of some of these problems, and we can be sure that new challenges will continue to evolve in the next millenium and the second century of the School's existence.

## PLAGUE

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Plague conjures up folk memories of massive disasters in the distant past and it was with disbelief that many people regarded the first reports of the Indian plague epidemic of 1994. Travellers took panic measures and health authorities searched for people who had any knowledge of the disease and its control. Although worldwide plague transmission had been at a relatively low level during the previous decades there was some increase in the number reported to WHO in the early 1990s, with an average of 2025 cases per annum [6]. Cases were recorded in Asia, Africa and the Americas each year.

Table 2. Selected websites related to exotic, emerging and re-emerging pathogens and travel health

| Site | Address |
|---|---|
| Liverpool School of Tropical Medicine | |
| general | http://www.liv.ac.uk/lstm/lstm.html |
| Travel Clinic | http://www.liv.ac.uk/lstm/travelmed.html |
| tropical links | http://www.liv.ac.uk/lstm/internet.html |
| London School of Hygiene and Tropical Medicine | http://www.lshtm.ac.uk/ |
| Royal Society of Tropical Medicine and Hygiene | http://www.rstmh.org/ |
| American Society of Tropical Medicine | http://www.astmh.org/index.htm |
| Centre for Comparative Infectious Diseases | http://www.pcweb.liv.ac.uk/vets/ccid_home.html |
| Eurosurveillance Weekly | http://www.eurosurv.org/ |
| World Health Organization | |
| (CRS = communicable disease surveillance and response) | http://www.who.int/emc/index.html |
| Centers for Disease Control (Atlanta) | |
| emerging infection links | http://www.cdc.gov/ncidod/id_links.htm |
| Emerging Infectious Diseases | http://www.cdc.gov/ncidod/EID |
| travel information | http://www.cdc.gov/travel/index.htm |
| Morbidity and Mortality Weekly Report | hhtp://www/2.cdc.gov/mmwr/ |
| National Institute of Allergy and Infectious Diseases, National Institutes of Health | |
| Emerging Infectious Disease Research | http://www.niaid.nih.gov/eidr/cover.htm |
| Clinical Tropical Medicine Links | http://medinfo.dom.uab.edu/geomed/links.html |

## Yersinia pestis

The cause of plague is a non-motile, relatively inert, gram-negative short rod. Yersinia pestis shows bipolar staining with Wayson or Giemsa stains and grows on blood agar to form pin-point sized colonies at 24 h. Y. pestis is a member of the Enterobacteriaceae and is very closely related to Y. pseudotuberculosis; indeed, it is regarded by microbiologists as a subspecies of that organism. Laboratories unused to dealing with plague are likely to mis-diagnose the infection as Y. pseudotuberculosis if they rely on some commercial systems and do not undertake appropriate biochemical tests [7]. Capsular fraction 1 antigen helps to prevent phagocytosis; it is present in the great majority of virulent strains and is the basis for most serological tests [8]. Three biotypes of Y. pestis differentiated on the ability to ferment glycerol and reduce nitrate are related to the three great pandemics of plague. Antiqua caused the sixth century epidemic in the reign of emperor Justinian which is thought to have killed 100 million people in the Mediterranean region. Medievalis caused the so-called 'black death' of the 14th century which killed at least a quarter of the population of Europe. Orientalis caused the pandemic of plague which started in southern China and spread to Hong Kong in 1894 and subsequently throughout much of the world. rRNA gene restriction patterns (ribotypes) correlate with the plague biotypes and may be useful for epidemiological studies [9].

## Epidemiology

Plague is a typical bacterial zoonosis. The reservoir of infection is in rodents and other small mammals. Their fleas are the principal mode of transmission. Some rodents are relatively resistant to plague and help to keep the focus active over long periods [10]. Y. pestis can also remain viable in rodent burrows and also in hibernating fleas for many months, sometimes as an L-form [11]. People may be infected by flea bites whilst walking through a zoonotic focus or hunters directly from the carcass of an animal whilst preparing it. Such infections are likely to be sporadic and uncommon.

The situation is much more dangerous when plague infects rodents living in close contact with human populations. The black rat Rattus rattus is the classic origin of human plague, as it likes to live in houses and to travel in ships. These rats are killed by plague infection and 'rat fall' is a typical precursor of a human epidemic. The brown or sewer rat R. norvegicus is not usually in such close contact with man and, where it has replaced R. rattus, plague is less likely to spread. Other rodents that come into close contact with man, such as the multimammate rat Mastomys natalensis, are also important spreaders of plague. Floods or unusual storage of grain in towns may result in greater contact between man and rodents and herald a plague outbreak.

Plague is most likely to be transmitted by fleas whose hypopharynx becomes blocked by masses of Y. pestis. Some species of fleas such as the oriental rat flea Xenopsylla cheopis are notorious for becoming blocked. A blocked flea will bite repeatedly to try to relieve its thirst, regurgitating plague bacilli into the wound with each bite. The 'human' flea Pulex irritans does not readily become blocked, but in populations with heavy flea infestations, as during the Middle Ages or today in the Andes mountains, these fleas may support transmission.

Pneumonic plague is the most feared form of the disease both because it is rapidly fatal and because droplet spread can cause an explosive epidemic. Primary plague pneumonia can result from the inhalation of bacteria directly from handling sick animals, e.g., in the Mongolian epidemic of 1910 where hunters of the tarabagan were infected and then passed on the plague as they huddled together in

underground tunnels to keep warm [12]. Domestic cats have proved to be the source of plague pneumonia on several occasions in the USA [13]. Secondary plague pneumonia during the course of bubonic or septicaemic plague can also initiate an outbreak of droplet-spread disease. Fortunately, most such outbreaks die out rapidly.

Very occasionally, people are infected by eating an infected domestic animal such as a goat or camel [14] or by biting a flea between their teeth. Scratches or bites by an infected cat or rodent are other unusual routes of infection, as is infection from pus discharged by a ruptured bubo.

## Clinical plague

The incubation period of plague is 1–7 days. There are three main clinical presentations – bubonic, septicaemic and pneumonic plague. Asymptomatic infections with plague can occur and give rise to positive antibody tests [15]. Y. pestis was cultured from the nasopharynx of asymptomatic contacts of patients with plague in Vietnam, but has not been shown to be transmissible.

Bubonic plague is the commonest presentation and is the usual result of an infected flea bite. It probably requires very few organisms to infect; $\leqslant$10 injected bacilli can kill a mouse [16]. Bubonic plague presents as an acute febrile illness, often with pain at the site of infected lymph nodes. Sometimes there is also diarrhoea and vomiting; but within a day or two there is an enlarged tender mass of lymph nodes, sometimes surrounded by oedema and most often in the inguinal region, but otherwise in the axilla, neck or elsewhere. There is usually no obvious source for the infection and no lymphangitis; rarely, a small nodule or ulcer can be found at the site of the flea bite and this may even resemble anthrax [15]. Most patients with bubonic plague are very ill and death rates in the absence of adequate treatment are often 50–60%. Some people may have a much milder attack and present with tender nodes but little or no fever – so-called ambulant plague or pestis minor; this is perhaps due to infection with less virulent strains of Y. pestis and tends to occur at the end of an epidemic. Bubonic plague in man is usually a dead end for the bacillus since efficient vector fleas do not often bite human beings and even if they do they are only likely to pick up bacilli if there is significant septicaemia. Dissemination can sometimes occur from ruptured buboes or from development of secondary plague pneumonia.

Septicaemic plague is essentially the severe end of the spectrum of bubonic plague. The presentation is an acute febrile illness often with vomiting and diarrhoea, abdominal pain, tachypnoea and very rapid progression to a moribund state. Pain in the inguinal region is sometimes present, but clinical diagnosis is difficult unless exposure to a plague focus is evident [17, 18].

Pneumonic plague probably requires the inhalation of an aerosol of 100–500 organisms [19]. It presents like a severe lobar or bronchopneumonia but progresses very rapidly to the expectoration of large quantities of watery blood-stained sputum with shock and sometimes ecchymoses. Once again the nature of the pneumonia may not be recognised unless the clustering of cases is appreciated [20]. There is a significant risk of spread to household contacts and attendants unless the patient is kept in respiratory isolation and the contacts receive chemoprophylaxis.

Plague meningitis is most often the result of inadequately treated bubonic plague, although it can be a primary presentation. It seems to be more likely in those with an extra-inguinal bubo. The presentation is similar to that of pyogenic meningitis [10].

## Diagnosis of plague

Blood cultures should be taken early before antibiotics are given, as there is usually an early bacteraemia in bubonic plague. Isolation may also be attempted from sputum, tracheal washings and especially lymph node aspirates. Blood films may show bacteria in septicaemic plague. Smears should be examined for bipolar staining and by immunofluorescence or with a fibre optic biosensor against the F1 antigen [17, 21]. PCR techniques are being developed and these helped to confirm the Indian plague epidemic [22–24].

## Treatment of plague

It is essential to start effective antibacterial treatment at once. Antibiotics are likely to be ineffective if started $>$1 day after symptoms develop in septicaemic or pneumonic plague. Streptomycin intramuscularly in divided doses totalling 30 mg/kg/day for 10 days is standard treatment. High-dose tetracycline or gentamicin are alternatives. Parenteral chloramphenicol is preferred for the treatment of plague meningitis because of better meningeal penetration [25]. Penicillins and cephalosporins have generally proved ineffective in man, although ceftriaxone is highly effective in vitro and in the mouse model [26, 27]. Fluoroquinolones also appear promising but have not been satisfactorily evaluated in man. Antibiotic resistance has not been a problem in human plague, although occasional strains resistant to tetracycline have been grown from rodents. A worrying development is the report of a highly resistant strain of Y. pestis cultured from a 16-year-old boy with bubonic plague in Madagascar during 1995. Resistance to ampicillin, chloramphenicol, kanamycin, streptomycin, spectino-

mycin, sulphonamides, tetracycline and minocycline was demonstrated. Resistance could be passed in vitro to other strains of Y. pestis by the conjugative plasmid pIPI202 [28]. Although human bubonic plague is generally a dead-end for the bacterium, if a resistance plasmid was widely distributed in the rodent reservoir, plague may prove difficult to treat in future. The threat of resistant plague as a weapon of biological warfare is also real.

## Immunisation against plague

Formol-killed, virulent Y. pestis vaccines have been used for many years and appear to provide useful protection in monkeys and man against bubonic plague, but may not be effective against pneumonic infection. Two or three doses of vaccine are needed in the primary course with 6 monthly booster doses in those still exposed. Live attenuated vaccines with Y. pestis EV 76 strains have also been widely used, but doubt remains about both their efficacy and safety [29]. With advances in molecular biology, new experimental vaccines are being produced. Monoclonal antibodies against the fraction 1 capsular protein (F1) of Y. pestis, or recombinant F1 vaccine, protect mice against pneumonic and bubonic plague infection with wild $F1^+$ strains [16, 30]. Vaccines should also be able to protect against strains that lack the F1 protein but may still prove virulent, although these strains are rare in nature. Subunit vaccines containing recombinant V antigen of Y. pestis protect mice against wild $F1^+$ or $F1^-$ strains [31, 32] and combined F1 and V subunit vaccines show promise [33, 34]. Whilst vaccines are useful in those occupationally exposed to plague, currently available vaccines are not of much use during a plague outbreak, because immunity is too slow to develop.

## Plague control

There is insufficient space to deal adequately with plague control in this article. Patients, their close contacts and their baggage must be dis-infested of fleas. Careful respiratory isolation is needed for anyone suspected of having pneumonic plague and the contacts of such patients should receive chemoprophylaxis with tetracycline for a week.

It is essential to kill fleas before attempting to kill rodents, or hungry, infected rodent fleas are more likely to bite people. Control of rodents in close contact with human populations by poisoning or trapping is then very important. It is not usually possible to eliminate plague infection in natural wild foci and education of the public who may walk or hunt in these areas is necessary so that infections are avoided or recognised early. Careful surveillance of plague foci is also needed.

# MELIOIDOSIS

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## Introduction

Knowledge about melioidosis progressed relatively slowly from its description by Whitmore and Krishnaswami in 1912 [35] until the 1980s. Over the past 15 years, the recognition of its importance as a public health problem, particularly in Thailand and northern Australia, has accelerated the pace of research on this fascinating disease and its causative organism, Burkholderia pseudomallei. This brief paper gives an overview of the disease with particular emphasis on recent developments and the risks of imported infection.

## Distribution and ecology

The worldwide distribution of B. pseudomallei was comprehensively reviewed in 1991 [36], since when there has been little substantial change. The main endemic areas are south-east Asia and northern Australia, although the true incidence is unknown throughout much of this region. This is at least partly due to the fact that melioidosis is a disease which usually affects the rural poor in areas where there is little access to the sophisticated diagnostic facilities needed to confirm the diagnosis.

In Thailand, it has been estimated that 2000–5000 cases occur each year [37]. The disease is commonest in the north-east of Thailand, for reasons that remain unclear. In Ubon Ratchatani province, the mean annual incidence of melioidosis seen in the provincial hospital was 4.4 (95% CI: 3.8–5.0)/100 000 population between 1987 and 1991 [38], and in Khon Kaen, it is the commonest cause of community-acquired pneumonia for which a cause is identified [39]. Perhaps surprisingly given the urban nature of the environment, 185 cases were notified in Singapore between 1991 and 1994, an overall annual incidence of 1.6/100 000 population [40], and a further 160 cases were diagnosed during 1995–1996. There is mounting evidence that melioidosis is endemic in China, particularly on the island of Hainan and the surrounding coastal provinces [41], and two indigenous cases of melioidosis have also been reported from Taiwan [42]. Evidence for the existence of melioidosis in India is also growing [43] and the subcontinent has been notable as a source of cases recently imported into the UK (see below).

Elsewhere in the world, the position is less certain. In northern Australia, B. pseudomallei is the commonest cause of fatal community-acquired bacteraemic pneumonia at the Royal Darwin Hospital, although a mere 20–30 cases were diagnosed in the Northern Territory

each rainy season between 1990 and 1996 [44]. Only a single case of caprine melioidosis has been described in Africa since 1991 [45], and two cases have been reported from the Caribbean [46, 47]. For the reasons described above, it remains likely that melioidosis is widely prevalent in the tropics but greatly under-diagnosed.

It is now well established that B. pseudomallei is an environmental saprophyte and not a zoonotic organism as was once thought. What are less well understood are the features of an environment that make it a suitable niche for B. pseudomallei. The disease shows evidence of patchy distribution and space–time clustering [38]. This is likely to reflect either differences between the susceptibilities of the indigenous population, variations in specific practices resulting in exposure to the organism, differences in virulence between local strains of B. pseudomallei, or variations in the concentration of B. pseudomallei in the soil, presumably related to some climatic, geological or ecological factors. The organism can be isolated from up to two-thirds of rice paddies in north-east Thailand [48], and it is present in significantly higher titres in soil from north-east than central Thailand [49]. The picture has been further complicated by the recognition that environmental isolates can be separated into two distinct biotypes, one of which is apparently avirulent and may ultimately be assigned to a separate species. Virulent isolates are negative for arabinose assimilation ($Ara^-$) in the API20NE and have a number of other biochemical differences from avirulent $Ara^+$ isolates [50], belong to distinct ribotypes [51] and are highly lethal for experimental animals [52], although they are indistinguishable by simple biochemical and serological tests and antibiograms. Although it is probable that climate, soil chemistry and interactions with other soil organisms are all important in determining the distribution of melioidosis, there is room for a great deal of further work on the ecology of the disease.

## Epidemiology and pathogenesis

Research on melioidosis epidemiology to date has been limited to simple descriptive studies. Recent work has served merely to confirm existing observations. In a study of 423 cases of culture-positive melioidosis diagnosed during a prospective study in Ubon Ratchatani, north-east Thailand, Suputtamongkol et al. observed a bimodal age distribution, with the lower peak occurring in the first 9 years of life and the highest age-specific incidence occurring at 50–59 years in women and 60–69 years in men [38]. In Singapore, however, the risk of melioidosis increased steadily with age, being maximal in the over 65s [40]. The male:female ratio of 1.5:1 in Thailand [38] contrasted with that of 5:1 in Singapore [40], probably reflecting differences in exposure to soil and water through rice farming, which was confirmed as an important risk factor in the Thai study. Interestingly, the group most at risk of melioidosis in Singapore appears to be military personnel, especially during their early training [53]. There is a suggestion from studies in Singapore that Indians may be more at risk of infection than Malays and Chinese, although further work is needed to confirm this [40, 53].

Melioidosis is thought usually to result from inoculation of mud or inhalation of fresh water, although these were reported in only 5.2% and 0.5% of cases, respectively, in Thailand [38]. Relatively few patients can identify a specific incident before they become ill, probably because rice farmers are continually exposed to infection and because of the variable incubation period of melioidosis, which may remain latent for weeks, months or years. The strong correlation between the quarterly incidence of melioidosis and the average rainfall is assumed to reflect seasonal exposure to the organism in the environment (e.g., during rice planting and harvesting) [38], although the possibility that other seasonal factors (e.g., dietary changes, stress, or concomitant viral infection) precipitate relapses of latent melioidosis cannot be excluded. Interestingly, there was no apparent correlation with rainfall in Singapore [40], suggesting that the rice farming activities themselves might be more important than rainfall per se.

As with most bacteria, the outcome of exposure to B. pseudomallei appears to depend on the balance between the virulence of the strain concerned and the immune function of the host. Clinically apparent melioidosis is an opportunist infection, 51–83% of cases having recognised underlying disease [38, 40]. The association with diabetes mellitus is particularly strong, and it has been suggested that the modulation of growth of B. pseudomallei by insulin which has been observed in vitro may play a role in this, although other immunological factors are probably also important [54]. Conversely, host cytokines may actually serve to cause disease manifestations [55]. The virulence determinants of B. pseudomallei are still poorly understood, despite increasing study in recent years. Several bacterial products are thought to contribute to pathogenicity, including lipopolysaccharide (LPS) [56], a lethal exotoxin [57], cytotoxins [58], various enzymes (lecithinase, lipase, proteases, acid phosphatase) [59, 60], extracellular polysaccharides [61] and a siderophore [62]. Intracellular survival of B. pseudomallei probably contributes to the recalcitrant nature of melioidosis [63, 64]. The scales can also probably be tipped in favour of disease by the size of the inoculum, as shown by the high attack rate in patients given contaminated injections (Dance, personal observations) or military recruits [53].

## Clincal manifestations and diagnosis

The clinical spectrum of melioidosis is extremely broad and has been repeatedly reviewed elsewhere, e.g.,

Dance 1998 [65]. Approximately 60% of patients are bacteraemic, and most of these present with community-acquired sepsis syndrome and evidence of multifocal metastatic infection, particularly involving the lung, skin and soft tissues, and liver and spleen, although any organ or tissue may be affected. The remaining 40% have localised infections, usually abscesses or granulomata, again most often in the lung, skin and soft tissues, liver and spleen, with parotitis accounting for nearly one-third of paediatric cases. New manifestations of melioidosis are being described regularly, of which the most interesting has been so-called 'neurological melioidosis'. This syndrome, which may be toxin-mediated, comprises peripheral motor weakness, brain-stem encephalitis, aseptic meningitis and respiratory failure [66].

In view of the non-specific nature of the clinical features, melioidosis is difficult to diagnose without laboratory support, and has been nicknamed 'the remarkable imitator'. The most reliable diagnosis is achieved by isolation and identification of the causative organism, which is invariably associated with disease. Serological tests may help to suggest infection when rising titres are seen or when very high titres are found, although high levels of seropositivity are found in populations in endemic areas. Several groups are developing assays to detect bacterial antigens and nucleic acids, although none of these has yet found a role in routine diagnosis in endemic areas, with the possible exception of immunofluorescence [67].

## Antibiotic susceptibility and treatment

B. pseudomallei is intrinsically resistant to many antibiotics, including most early $\beta$-lactams and the aminoglycosides. Until the 1980s, empirical combination regimens comprising chloramphenicol, tetracyclines and co-trimoxazole were used to treat melioidosis. There have now been three large published randomised trials of the treatment of acute, severe infections. In the first of these, ceftazidime (120 mg/kg/day) was shown to reduce the mortality of patients with culture-positive melioidosis from 74% to 37% compared with conventional combination treatment [68]. In a second study, which differed in using a slightly lower dose of ceftazidime (100 mg/kg/day) in combination with co-trimoxazole, the overall mortality was somewhat lower, but ceftazidime treatment was again associated with a significantly reduced mortality for culture-positive disease (18.5% compared with 47%). Unfortunately, differences in disease severity between the groups may have affected the results in this latter trial [69]. It is also unfortunate that ceftazidime with and without co-trimoxazole has not been compared in order to determine whether there are any differences between acute mortality, relapse rates and the development of antibiotic resistance. Finally, parenteral ceftazidime has been compared with parenteral co-amoxiclav [70]. Although the overall mortality in each group was equivalent in this study (47%), more patients treated with co-amoxiclav were classified as 'treatment failures', requiring a change of therapy, and so ceftazidime, with or without co-trimoxazole, remains the treatment of choice. Parenteral treatment should be given for at least 2 weeks, and sometimes longer in patients with extensive abscesses who fail to defervesce on treatment. Obviously, adjunctive treatment (e.g., correction of volume depletion, management of renal impairment and metabolic disturbances, drainage of abscesses, etc.) may be required.

Following parenteral treatment, there is a considerable risk of relapse or, rarely, re-infection [71]. This may be as high as 23% [72], although prolonged (20-week) courses of oral antibiotics can reduce the risk to $< 5\%$ [73]. Conventional agents are associated with a lower risk of relapse, although co-amoxiclav should be used in children or pregnant women.

Unfortunately, the treatment of melioidosis with these newer agents is very expensive, and this will limit their use in the poor rural areas of the tropics where melioidosis is endemic. Sadly, there are no new agents on the horizon that are likely to be more effective or more affordable. The carbapenems have some theoretical advantages over ceftazidime [74], but if anything they are more expensive. Fluoroquinolones have an unacceptable failure rate [75]. Efforts will have to go towards prevention of infection, either through avoidance of exposure to the organism – especially for high-risk individuals such as diabetics – or through the development of vaccines [76].

## Imported infections

The risks of imported infection are small, but increasing as ever more people go on holidays to exotic destinations, and as the population of immigrants from endemic areas increases. The cases known to have been diagnosed in the UK during the past 10 years are summarised in Table 3. Notable features include the proportion of cases acquired in the Indian subcontinent, the potentially long incubation period, and the importance of diabetes as a predisposing factor. The laboratory should always be alerted if melioidosis is suspected to enable appropriate selective techniques and containment measures to be used.

Melioidosis may also be imported by other species, e.g., feral cynomolgus monkeys, from endemic areas [77]. Although such events are unlikely to present a threat to human public health, the example of an outbreak of melioidosis in France during the mid-1970s suggests that we cannot afford to be complacent [78].

Table 3. Melioidosis diagnosed in the UK 1988–1998

| Year | Sex | Age (years) | Origin | Latency | Underlying disease | Died |
|---|---|---|---|---|---|---|
| 1988 | M | 60 | Pakistan | >2 years | DM | Yes |
| 1989 | M | 40 | Malaysia | – | Nil | No |
| 1990 | M | 54 | Bangladesh | >6 months | DM | Yes |
| 1992 | M | 53 | Thailand | 4 weeks | DM | Yes |
| 1994 | M | 58 | Bangladesh | – | TB | No |
| 1995 | M | 55 | India | 3 weeks | DM | Yes |
| 1996 | F | 51 | Thailand | – | Steroids | No |
| 1997 | M | 61 | Thailand | – | Nil | No |
| 1997 | M | 49 | Indonesia | – | DM | Yes |
| 1998 | M | 20 | Malaysia | >2 years | CF | No |

M, Male; F, Female; DM, diabetes mellitus; CF, cystic fibrosis; TB, tuberculosis.

## CHOLERA: AN OLD DISEASE – A NEW EPIDEMIC

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Cholera is one of the oldest recorded infectious diseases, and cholera epidemics and pandemics have been closely associated throughout history with wars, population movements and natural disasters. Accounts of a cholera-like illness before the nineteenth century range from descriptions in early Greek, Sanskrit and Chinese writings, to the writings of travellers in Europe and Asia in the sixteenth and seventeenth centuries. The recent history of cholera began in 1817, with the first of six pandemics that occurred from 1817 to 1923 (Table 4). During the nineteenth century, important advances in the understanding of cholera arose from the epidemiological studies of John Snow, who demonstrated spread by infected water, bacteriological studies by Pacini in 1854, who first described curved bacilli in the intestinal contents of cholera victims, and Robert Koch, who in 1883 demonstrated that cholera was caused by the comma-shaped bacillus, Vibrio cholerae [79].

From 1926 until the beginning of the seventh pandemic in 1961, outbreaks of cholera continued in the Indian subcontinent, the Middle East, south-east Asia and China.

In the last two decades, although more than a century has elapsed since the cause of cholera and its mode of transmission were determined, and despite international efforts to improve water supply and sanitation, cholera has become an increasing public health problem in much of the tropical and less developed areas of the world [80].

### Bacteriology and pathogenesis

V. cholerae is a gram-negative, comma shaped bacillus that ferments glucose with the production of acid but no gas, and is oxidase positive. The species contains many serotypes, based on differences in the O surface antigen oligosaccharides. Until 1992, only serotype O1 caused the disease defined, epidemiologically and clinically, as cholera. Serotype O139/Bengal is described later. The O1 serotype is divided into three antigenic subtypes, Inaba, Ogawa and Hikojima. These subtypes are characterised by variations in the composition of the O1 antigen, but have not been fully characterised. Both in the laboratory and during epidemics, there may be antigenic shifts between Inaba and Ogawa. V. cholerae O1 is also divided into two biotypes, classical and El Tor. El Tor was first described in 1904 in the Sinai peninsular, but did not become of global importance until the seventh pandemic that began in 1961 [81].

The pathogenesis of cholera, and particularly cholera toxin, have been well described, but recently additional factors involved in pathogenesis have been identified. Cholera enterotoxin was first described by De, in Calcutta in 1959 [82]. The toxin consists of two polypeptide subunits, A and B. The B subunit comprises five peptides, each with a mol. wt of 11 604 Da, arranged in a radial fashion at the end of the linear A subunit. The A subunit comprises two parts, A1 with a mol. wt of 21 817 Da and A2 with a mol. wt of 5398 Da. In summary, the B subunit binds to

Table 4. The first six cholera pandemics

| Pandemic | Date | Indian subcontinent | South-east Asia | Middle East | Europe | North Africa | East Africa | Americas |
|---|---|---|---|---|---|---|---|---|
| First | 1817–1823 | + | + | + | ... | ... | + | ... |
| Second | 1826–1837 | + | + | + | + | + | + | + |
| Third | 1842–1862 | + | + | + | + | + | + | + |
| Fourth | 1865–1875 | + | + | + | + | + | + | + |
| Fifth | 1881–1896 | + | + | + | + | + | + | + |
| Sixth | 1899–1923 | + | + | + | + | ... | + | ... |

ganglioside (GM1) receptors on the epithelial cell membrane of the small intestine. Binding of the B subunit allows the A subunit to enter the epithelial cells and activate adenylate cyclase. This enzyme mediates the transformation of ATP to cyclic AMP (cAMP) resulting in decreased absorption of sodium and chloride ions, active chloride and bicarbonate secretion, and the massive loss of fluid and electrolytes that characterises cholera.

Additional toxins and other factors are now known to be involved in cholera pathogenesis. Zonula occludens toxin (Zot) was described by Fasano et al. in 1991 [83]. Zot increases the permeability of the small intestinal mucosa by affecting the structure of the intercellular tight junctions. Accessory cholera exotoxin (Ace toxin) was described by Trucksis et al. in 1993 [84]. Ace toxin is related to increased trans-membrane ion transport. Colonisation factors, in particular the pilus colonisation factor TCP, are necessary for the adherence of V. cholerae to intestinal epithelial cells, and strains deficient in the gene coding for TCP have been shown to be non-virulent [85].

The genetic basis of many of the cholera virulence factors has been determined in recent years. The genes for the A and B subunits of cholera enterotoxin, ctxA and ctxB, are arranged on a single transcriptional unit. The synthesis of cholera toxin is positively regulated by the gene toxR [86]. The ctx/toxR operon, together with genes coding for Zot and Ace, is located on a 4.5-kb region of the chromosome called the core region. The toxR gene appears to play a crucial role in the expression of virulence factors other than cholera toxin, including the colonisation factor TCP, outer-membrane protein and lipoproteins [87]. The regulatory activity of toxR may be influenced by environmental signals, including osmolarity, pH and temperature.

The increased understanding of the genetics of V. cholerae has provided important ways of improving studies on cholera ecology and epidemiology, on the development of improved cholera vaccines, and investigating the spread of serotype O139.

## Cholera ecology and epidemiology

There has been considerable recent debate over the role of the environment and human carriers as reservoirs for cholera in the absence of symptomatic cases. Non-O1 V. cholerae is part of the normal, free-living bacterial flora of estuarine and other waters. V. cholerae O1 can survive for weeks to months in the natural aquatic environment, but there is uncertainty as to whether this occurs only in relation to frequent contamination by infected people [88] or whether V. cholerae O1 truly survives as an environmental bacterium. Recent studies have helped to explain these different results by the description of ‘non-culturable’, dormant strains, which may persist for long periods in natural aquatic environments [89]. Change from the dormant to a culturable form may be influenced by environmental factors and may involve environmental regulation of the toxR gene. Within the aquatic environment, V. cholerae has a close ecological relationship with crustaceans and some aquatic plants, and their persistence and seasonality may also influence the occurrence of V. cholerae. V. cholerae may also exist as a ‘rugose’ form, associated with the production of an exopolysaccharide in which many bacteria may be aggregated [90]. Rugose variants appear to be resistant to chlorine, and may survive despite water chlorination treatment [91].

While long-term human asymptomatic carriage and excretion of V. cholerae have rarely been recorded, persistence within a community by low-level, asymptomatic transmission is probably the major way of maintaining infection in endemic areas [92].

The seventh pandemic of cholera (Fig. 2) began in 1961, originating on the island of Sulawesi in Indonesia. The pandemic strain was V. cholerae O1 El Tor, and it spread rapidly to neighbouring countries of south-east Asia. Between 1963 and 1969 the pandemic spread to the Indian subcontinent, displacing the classical biotype, and by 1970 had reached the Middle East. The pandemic strain entered Africa by two routes, in west Africa probably via a returning traveller, and from the Arabian peninsula, through Djibouti, Somalia into east Africa [93]. By 1978, most countries of central and southern Africa were affected. The final stage of the spread of this pandemic was the arrival of cholera in the South American continent in January 1991, the first time that cholera had entered the continent since the fifth pandemic in the 1880s [94]. The pandemic began in the coastal region of Peru, in three distinct foci. While the source of this introduction has not been proven, a possible source may have been ballast water in a freighter from Asia, discharged off the Peruvian coast. The pandemic rapidly spread to Ecuador and Colombia and by 1992 had extended both southwards and into central America.

With the exception of the possible introduction via ballast water into South America, the transmission of cholera from region to region is probably by short-term asymptomatic carriers. Local spread within communities, whether during epidemics or in periods of endemicity, is by the faecal–oral route through water, food, or contamination of utensils and other household items. Water may be contaminated at source, either in areas where untreated water sources are contaminated [95], or where municipal supplies are inadequately treated and become contaminated, which has occurred in some South American towns [96]. Contamination of water stored in the household has been demonstrated in studies in South America and India [97]. Sea-foods are a particularly important vehicle of transmission in areas where V. cholerae O1 can exist in the aquatic

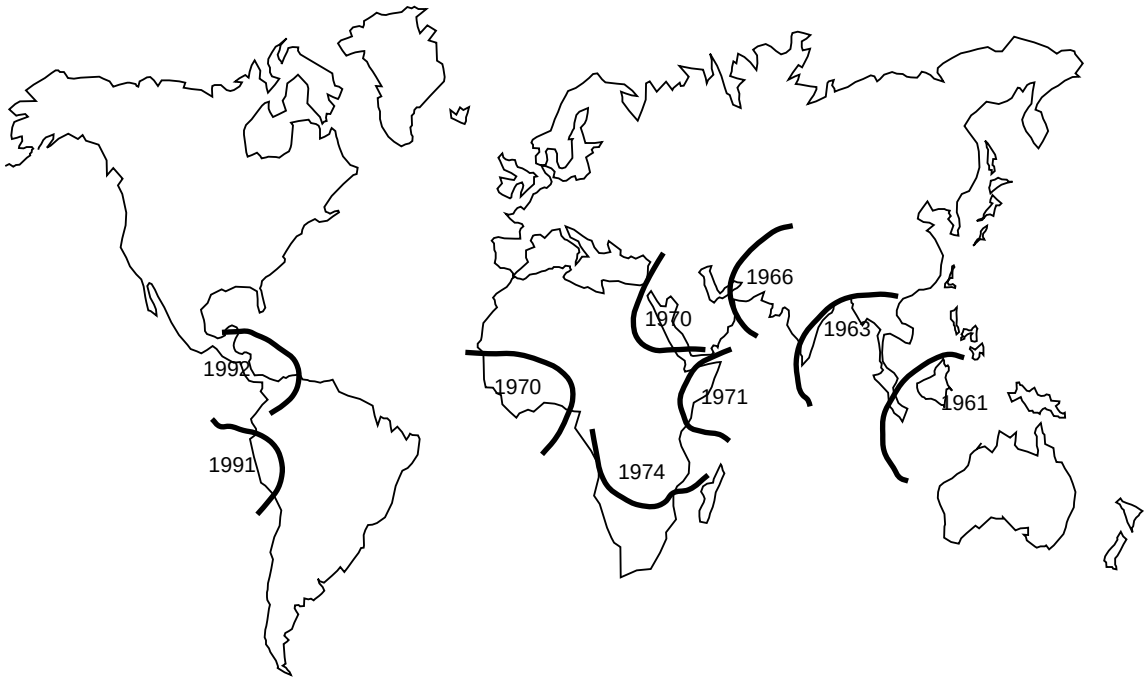


Fig. 2. The spread of the seventh cholera pandemic.

environment. Spread of cholera in parts of the South American epidemic was related to the contamination of fresh vegetables by contaminated water [98]. Contamination of food by asymptomatic excretors is responsible for spread in households and villages [99].

## V. cholerae O139

In October 1992 an outbreak of cholera occurred in south India caused by a new serotype of V. cholerae [100]. While non-O1 vibrios had been responsible for isolated cases of diarrhoea, this was the first report of epidemic cholera caused by a non-O1 strain. Non-O1 strains had been categorised into 137 serotypes, from O2 to O138, hence the designation of the new serotype O139. V. cholerae O139 subsequently spread through India to Bangladesh, and isolates have been reported from Thailand to central Asia. V. cholerae O139 appears to be derived from the El Tor biotype, with both ctxA and ctxB genes, but differs from O1 serotypes in producing a polysaccharide capsule. The current epidemiology of O139 is somewhat complex. After its initial appearance in Bangladesh in 1992, it gradually became the dominant serotype, apparently displacing V. cholerae El Tor O1 and becoming established in the aquatic environment. However, since 1994, El Tor O1 has again become the dominant serotype, although genetic studies of the present El Tor strains showed differences in chromosomal DNA restriction digest patterns compared with those isolated before the appearance of O139 [101].

As previous exposure to V. cholerae O1 will not provide bactericidal antibodies to O139, populations in O1 endemic areas will not be immune, and the spread of O139 has the potential to begin a new pandemic.

## Cholera immunity and vaccine development

Immunity to both cholera toxin and bacterial surface antigens is necessary for protection against disease, and follows natural infection. Several studies have suggested that antibacterial immunity plays a greater role than antitoxin immunity [102]. While most studies of immune response measure serum vibriocidal antibody, protection in vivo is more likely to be mediated by secretory IgA. The dominant bacterial antigen is the LPS component of the cell wall, but recently additional immunogenic molecules have been described, including flagellum protein, cell-associated haemagglutinins and outer-membrane proteins [103].

Until the 1980s, only parenteral killed whole-cell cholera vaccines were available. Controlled trials in the 1960s in cholera endemic areas demonstrated an efficacy of only 30–60% for parenteral vaccines, and no reduction in carriage rates [104]. These vaccines are no longer recommended by the WHO.

Orally administered vaccines, to produce antitoxin and antibacterial intestinal immunity, now form the basis of vaccine development. Two different vaccine types are currently under investigation and trial. Both strategies include whole bacteria to produce antibacterial immunity, and the B subunit toxin, which is necessary for entry of the A subunit into epithelial cells, but is itself not pathogenic. Antibodies to the B subunit will, on exposure to infection, block the B subunit and so prevent intracellular entry of the A subunit.

One strategy has been to use killed V. cholerae cells and purified B subunit toxin, the WC/B vaccine. Extensive trials in Bangladesh showed up to 85%

protection, although protection declined after the first year, and was considerably less in children [105]. Oral administration of a locally produced, whole-cell vaccine without the B subunit was tested in a large field trial in Vietnam, giving a protective efficacy of 60% [106].

The second strategy has used molecular techniques to produce attenuated V. cholerae, deficient in the A subunit, by removing the ctxA gene. An early vaccine of this type was the Texas Star–SR vaccine, in which the ctxA gene was deleted by chemical mutagenesis. Molecular techniques were subsequently used to selectively delete the ctxA gene sequences, and the strain CVD 103-HgR is the most advanced example of this strategy. The strain, which is derived from the classical biotype, has produced 80–100% protection against challenge with classical strains and 60–70% protection against El Tor [107]. While most molecular work has been on the classical biotype, an El Tor-based, live attenuated oral vaccine has been derived recently from Peruvian strains (Peru-14 and Peru-15) [108].

Although the development of these new vaccines may have an important contribution to play in the prevention of cholera in particular epidemiological settings, they are unlikely to be effective in protecting against O139, as the bactericidal antibody is serotype-specific.

### Cholera in Africa: an expanding epidemic

While the re-introduction of cholera into South America in 1991 and the appearance of serotype O139 in Asia in 1992 have been of great public health importance in the present decade, the epidemic in Africa since 1994 has been a catastrophe. In 1994, the largest proportion of all cholera cases globally, and 42% of all cholera deaths, were in Africa. This was largely due to the explosive epidemic in Rwandan refugees displaced to eastern Zaire, where over a 6-week period there were an estimated 70 000 cases and up to 12 000 deaths [109]. A pandemic situation has continued in this central area of Africa, with both extensive endemic spread and explosive localised epidemics in Zaire, Burundi, Tanzania and Congo (Brazzaville) [110]. In west Africa, a major epidemic in Guinea-Bissau that began in 1994 has continued, with over 20 000 cases reported in 1997. In east Africa, excessive rains and flooding in 1997 were followed by extensive outbreaks of cholera in Somalia and Kenya. In the African epidemics, mortality rates from cholera have ranged from 5% to 15%, compared with $< 2\%$ in most of Asia and South America.

WHO reports in the first part of 1998 [111] suggest that these epidemics are continuing, with little prospect of successful control through immunisation [112] or, at least in the short term, effective improved water supply and sanitation strategies.

It is more than a century since both the epidemiology of cholera and the causative agent were described. Despite advances in immunisation and public health, cholera remains an epidemic disease of global concern, particularly in tropical and poorer countries. Both short-term strategies, for improved disease surveillance and early interventions in outbreaks, and longer-term studies to develop effective vaccines and understand the complex epidemiology of cholera are required if successful control is to be achieved.

## HUMAN EHRLICHIOSES

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The ehrlichiae are primarily animal pathogens that may occasionally infect man. The genus Ehrlichia was named in 1945 in honour of Paul Ehrlich and is part of the family Rickettsiaceae. They are obligate intracellular bacterial pathogens primarily replicating in parasitophorous vacuoles of either peripheral blood monocytes or granulocytes. Most are transmitted from animal to animal or from animal to man by hard-bodied ixodid ticks which act as biological vectors [113, 114]. They are divided into genogroups on the basis of 16S rRNA operon sequences, although these divisions do not always reflect subdivisions according to vector, host target cell or disease manifestation [113, 114]. In general, the infections can be divided into monocytic and granulocytic ehrlichioses depending upon the host cellular target (Table 5).

### Monocytic ehrlichioses

Until recently the only known human ehrlichiosis was Sennetsu fever. E. sennetsu was first described in Japan in 1955 [115] as a cause of infectious mononucleosis. It falls into genogroup III and like E. risticii, the cause of Potomac fever in horses, its cell division inside parasitophorous vacuoles stimulates expansion and division of the vacuole itself [116]. Sennetsu fever is believed to be transmitted by ingestion of raw fish and sporadic cases are reported from Japan and Malaysia.

E. canis, like the remaining monocytic erlichiae, is in genogroup I. It causes canine ehrlichiosis which has a worldwide distribution [113, 117–120]. It is transmitted by the brown dog tick (Rhipicephalus sanguineus) [121]. Following experimental infection E. canis can be detected in blood, spleen or bone marrow of two-thirds of the infected dogs 3–4 months later, thus indicating a ‘carrier state’ [122]. The first cases of human monocytic ehrlichiosis (HME), described in 1986, were thought to be due to E. canis [123]; the

Table 5. Monocytic and granulocytic ehrlichioses

| Ehrlichiosis | Genogroup | Transmission | Vector | Principal hosts | Geographic distribution |
|---|---|---|---|---|---|
| Monocytic | | | | | |
| E. canis | I | Tick bite | Rhipicephalus sanguineus | Canines (man) | Worldwide |
| E. chaffeensis | I | Tick bite | Ambylomma americanum | Deer, man, (dogs) | USA |
| E. muris | I | Unknown | ? | Mice, voles | USA |
| E. sennetsu | III | Ingestion of raw fish | – | Fish, man | Japan, Malaysia |
| E. risticii | III | ? Arthropods | – | Equines, canines, felines | USA |
| Granulocytic | | | | | |
| E. phagocytophila | II | Tick bite | Ixodes ricinus | Sheep, cattle, equines, dogs, deer, (man) | Europe |
| E. equi | II | Tick bite | I. pacificus, I. ricinus | Equines, canines | Mainland Europe, USA |
| E. microti | II | Tick bite | I. scapularis | Voles | USA |
| HGE | II | Tick bite | I. scapularis, I. pacificus, | Man, deer | USA, Europe |
| | | | I. ricinus | Rodents | USA |
| LGE | II | Tick bite | I. pacificus | Llama | USA |
| E. ewingii | I | Tick bite | A. americanum | Canines | |

HGE, agent of human granulocyte ehrlichiosis; LGE, agent of llama granulocytic ehrlichiosis.

causative organism was later shown to be E. chaffeensis [113]. Similar cases were subsequently described after exposure to ticks [124]. However, a recent case of HME in Venezuela has been ascribed to an agent more closely resembling E. canis than E. chaffeensis [125]. In 1991 a 'new' Ehrlichia spp. was cultured [126] from the blood of a military recruit based at Fort Chaffee in Arkansas, who had suffered a tick bite. He presented with fever and headache. The bacterium, the cause of HME, was named E. chaffeensis [126, 127]. E. chaffeensis and E. canis are closely related at both the genetic (16S rRNA) and antigenic level. However, there are differences, e.g., at the groESL heat shock operon and quinolinate synthetase A (NAD A) genes [128, 129]. For example, at the amino-acid level the NAD A genes of E. chaffeensis and E. canis have only 89.2% homology [129]. Such differences have provided evidence that dogs can also be infected with E. chaffeensis [130]. There are antigenic and genetic differences among various isolates of E. chaffeensis [131–133], but as yet none has been related to differential pathogenicity [132]. However, all isolates examined thus far express an immunodominant 28-kDa outer-membrane protein encoded on multicopy genes [134]. Six copies of the p28 gene were found to be arranged in tandem with intergenic spaces, but each gene contained a semivariable and three hypervariable regions, indicating a great propensity for antigenic variability. Antisera raised against recombinant p28 cross-reacted with E. canis [134].

The major reservoir of E. chaffeensis is the white-tailed deer [135], although it has been detected in dogs [130] and under experimental conditions persistent infection can be established in mice [136]. E. chaffeensis has been detected in the lone star tick (Amblyomma americanum) and the American dog tick (Dermacentor variabilis), but most cases of HME have been associated with A. americanum bites. Most cases of tick-borne HME have been reported from the USA, although cases have been described in Venezuela [125], Portugal [137], Belgium [138] and Mali [139], which suggests the possibility of alternative tick vectors. In the USA the distribution of HME mirrors the distribution of the tick vector. Although HME has been detected in >20 states, most cases are found in Texas, Oklahoma, Arkansas, Missouri and Georgia [140]. In Georgia, a study of febrile hospitalised patients [141] indicated that the prevalence of HME was 5.7 cases/100 000 population/year (similar to the incidence of meningococcal disease in England and Wales!). In a retirement community of avid golfers in rural Tennessee the annual rate of infection was found to be 660/100 000 population [142]. HME is predominantly rural (66% of cases) and seasonal (68% of cases). It occurs in older patients (median age 44 years), but has also been described in 37 children [140] and has a male preponderance [113, 140–142].

The incubation period varies from 2 days to 2 weeks from the tick bite [140], and presents with symptoms and signs of a systemic illness [123, 124, 140–142]. The most common findings are of headache (60–80% of cases), fever (100%), myalgia (65%), anorexia/nausea (60–70%), abdominal pain (50%) and hepatosplenomegaly (40%). A macular, maculopapular, petechial or mixed rash is described more frequently in children (66% of cases) than adults (<50%). In most cases the infection resolves uneventfully, but up to a quarter of hospitalised patients with HME are admitted to an intensive care unit [140] and HME does have a measurable mortality (2–3%). Severe complications include respiratory and renal failure and neurological complications including fits and fourth cranial nerve palsy [143].

The pathogenesis of HME is poorly understood, but E. chaffeensis is taken into monocytic early endosomes that do not fuse with lysosomes [144]. The early endosomes accumulate transferrin receptors, a process

that is up-regulated by the bacterium. Interestingly, E. chaffeensis-infected human monocytes did not express tumour necrosis factor (TNF), interleukin 6 (IL-6), or granulocyte macrophage colony-stimulating factor, but production of interleukins 1, 8 and 10 was up-regulated [145]. However, when the bacterium was pre-treated with human antisera, TNF, IL-1 and IL-6 were released in large amounts [146].

The diagnosis of HME is based upon the detection of antibody to E. chaffeensis (or E. canis) by immunofluorescence, of morulae in peripheral blood mononuclear cells, bacterial culture [126], or PCR amplification of the 16S rRNA operon [113]. A combination of these approaches provides the most sensitive diagnostic approach. Other laboratory findings include leucopenia (60–70% of cases), thrombocytopenia (70–80%) and elevated aspartate aminotransferase (85–95%). Treatment is with tetracycline (500 mg qds) or doxycycline (100 mg bd in adults or 4 mg/kg/day in two divided doses in children). Chloramphenicol (75 mg/kg in four divided doses) can be used in young children, but may be associated with treatment failures [140]. Treatment should be continued for 7–10 days. Prevention is by avoidance of tick-infested areas, but wearing insect repellent is also associated with a decreased risk of HME [142]. There is no vaccine available and, ominously, the immunodominant 28-kDa antigen shows considerable variability [134]. Prior infection of dogs with E. canis does not necessarily provide protection against subsequent challenge [147].

## Granulocytic ehrlichioses

The granulocytic ehrlichiae have been reviewed in a previous report [114], therefore this section will discuss only recent information. The genogroup II granulocytic ehrlichiae, E. equi, E. phagocytophila, E. microti and the agents of human granulocytic ehrlichiosis (HGE) and llama granulocytic ehrlichiosis (LGE) are very closely related genomically (by 16S rRNA and groESL genes) and antigenically [114]. Furthermore, cross-species transmission can occur naturally or under experimental conditions, indicating that they are conspecific. In contrast there are considerable genomic and antigenic differences between the granulocytic ehrlichiae and E. chaffeensis [148]. E. ewingii is in genogroup I and is the cause of canine granulocytic ehrlichiosis [149]. However, recent data suggest that E. equi has also caused granulocytic ehrlichioses in dogs in Italy [150] and the USA [151]. E. ewingii differs from the other granulocytic ehrlichiae in being transmitted by A. americanum. The rest are transmitted by ixodid ticks and it has been shown that Amblyomma species are not vectors of HGE [152]. Ticks may wait for months between blood meals. During this period tick-borne pathogens such as Rickettsia rickettsiae [153], Borrelia burgdorferi [154] and Babesia microti [155], remain dormant and are non-infectious until some hours after the tick has started its next blood meal. The same is apparently true for HGE, as in experimental murine transmission very few mice were infected if ticks were removed before attachment and feeding for 36 h [156]. A similar reactivation period (>24 h) has been described for E. phagocytophila [157]. Interestingly, host immunity to ticks appears not to prevent transmission of granulocytic ehrlichiosis [158] as it does for Lyme disease [159]. E. phagocytophila genogroup ehrlichiae have been detected in I. scapularis in eastern and mid-west USA, in I. pacificus in California [160] and in I. ricinus and I. trianguliceps in UK [161] and Italy [162]. Ixodid ticks will feed on almost any vertebrate host and it is probable that the ecology of HGE in the USA involves a deer tick–rodent cycle [163]. Also, granulocytic ehrlichiae are highly prevalent in white-tailed deer [164]. Rodent hosts for granulocytic ehrlichiae include Peromyscus boylii, P. manicalatus, P. glossypinus, Neotoma fuscipes (woodrat), N. lepida, N. albigula and N. mexicana [165]. However, a large number of other mammals including horses, dogs, goats, sheep, mice and cattle can be infected naturally or experimentally. Very rarely, transmission can occur by means other than tick bite, e.g., transplacental transmission of E. phagocytophila has occurred in cattle [166] and there is one reported case of perinatal transmission of HGE [167]. Although HGE is reported predominantly from the USA, infection has been detected in Sweden, Slovenia [reviewed in ref. 113] and in the UK [167, 169], Norway [170], Italy [171] and Switzerland [172]. It is also a cause of saddleback fever [173].

The primary site of replication of the granulocytic ehrlichiae is the mature neutrophil. This cell has a very short half-life (6–12 h), considerably less than the doubling time of most other obligate intracellular bacteria. Neutrophils die by apoptosis [174] and are removed from the circulation intact, without releasing their cytoxic granule contents, by macrophages [175]. Granulocytic ehrlichiae must somehow reverse neutrophil apoptosis to permit their replication and it is noteworthy that some bacterial metabolites such as butyrate can reverse apoptosis [176]. Neutrophils signal to a large number of other cell populations by releasing cytokines and chemokines [177]. Whether and how granulocytic ehrlichiae modulate this process is unclear.

The clinical features of HGE are very similar to those of HME with fever, chills, myalgia and headaches [114, 140, 178]. The mortality rate is c. 5%. In sheep E. phagocytophila infection causes an immunosuppression predisposing the animal to pyaemia, louping ill and clostridial diseases [114]. A similar increased susceptibility to other infections has been reported with HGE [114]. The diagnosis, treatment and prevention of HGE are the same as for HME.

## ANTIBIOTIC RESISTANCE IN THE TROPICS

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In recent years the global spread of bacteria resistant to many different antimicrobial agents has been dramatic. Whilst the impact of resistant bacteria has been considerable in the developed world it could be argued that it has been greater in tropical areas. In such areas infectious diseases are the principal cause of morbidity and mortality and any decrease, no matter how small, in the efficacy of antibiotics leads to a disproportionate decrease in the standards of individual and public health. The newer antimicrobial agents, to which such antibiotic-resistant strains are susceptible, may be unavailable because of their high price. Because of pressures on the public health and hospital infrastructure, cross-infection is a major problem, leading to the rapid dispersal of antibiotic-resistant bacteria. The spread of any resistant strains of bacteria is also assisted by increasing overcrowding of populations in developing countries due to the move to greater urbanisation. Political instability and conflict leading to the creation of large populations of refugees not only provides for the movement of large bodies of people across frontiers, but refugees are also particularly prone to infectious diseases, providing another route for dissemination of antibiotic-resistant bacteria.

The faecal flora is a large potential reservoir for antibiotic resistance genes and, as most antibiotics are given via the oral route, resistance is selected not only in enteric pathogens but also in commensal bacteria. In tropical countries, disposal of faeces is often poorly controlled and spread of such bacteria via direct contact, water or food is common. A detailed study of 20 healthy, small children over a 13-week period in a peri-urban community in Mexico City showed frequent and persistent shedding of multi-drug-resistant Escherichia coli. At some point during the study 88.5% of children carried a strain of E. coli resistant to at least two antibiotics [179]. In 1987, a similar study in the Sudan also found evidence of high carriage rates of antibiotic multi-resistant E. coli in healthy children [180].

In addition to the faecal reservoir of antibiotic-resistant bacteria, the upper respiratory tract commensal and opportunist flora represents another reservoir for resistance genes. Infections of the respiratory tract are an increasing problem in many urban areas of the tropical world. Huge increases in air pollution caused by automobiles and industry, combined with heavy promotion of tobacco smoking by multi-national tobacco companies, are all contributing to rises in respiratory infection. Overcrowding also means that opportunist respiratory pathogens can move freely within a susceptible population. Increased mobility of people throughout the world is also increasing the risk of pandemic spread of resistance genes arising in tropical areas [181]. This has been graphically illustrated in a number of reports, notably the importation on at least four separate occasions of E. coli simultaneously expressing three different $\beta$-lactamases (TEM-1, SHV-5 and BIL-1) from the northern part of the Indian subcontinent (ISC) into the UK. These strains had evolved in the ISC and were resistant to all clinically used $\beta$-lactam antibiotics (including $\beta$-lactamase inhibitors) with the exception of carbapenems [182]. In a study of the worldwide distribution of plasmid variants encoding tetracycline resistance in Neisseria gonorrhoeae using isolates obtained from travellers who acquired gonorrhoea in a number of tropical countries upon their return to the UK, the worldwide distribution of the Dutch and American plasmid variants was mapped [183]. Therefore, tourists and business men are distinct potential disseminators of newly evolved resistance genes. Finally, the movement of food-stuffs must constitute an important route in the future for the dissemination not only of pathogens more frequently encountered in tropical regions (e.g., Salmonella typhi, Vibrio cholerae), but also of coliforms carrying recently evolved resistance genes. Therefore, the increasing multi-cultural integration of different ethnic groups in the world will bring together antibiotic resistance genes that have evolved in very different ecosystems. Sometimes, the route of transfer of such bacteria is not always obvious. During the recent outbreak of cholera in Peru, shellfish in the southern sea coast of the USA were found to be contaminated with the same strain. This was traced to the discharge of ballast water from ships travelling from Peru to the gulf of Mexico.

The genetic mechanisms for the dissemination of antibiotic resistance genes amongst bacteria are reasonably well understood. Most studies have been carried out in hospital environments in the developed world merely because of the availability of facilities for extensive culture, not only of bacterial pathogens, but also the co-existing commensal flora and the ability to undertake a detailed molecular analysis of the resistance determinants.

Few similar studies have been undertaken in the developing world, which is unfortunate in that the relative contribution of different drivers of resistance could well be different. Many resistance genes are located on the bacterial genome and have arisen by mutation of the target genes (e.g., gyrA mutations giving rise to fluoroquinolone resistance and rpoB for resistance to rifampicin in Mycobacterium tuberculosis). These genes can be mobilised by transposition, recombination or via integrons (DNA sequences with a single recombination site capable of movement and expression in widely differing hosts [184]) on to plasmids which can then move between different bacterial species and genera. An excellent example of such an event occurring in a tropical country is that

reported by Frost and colleagues in 1985. Analysis of isolates of Shigella dysenteriae that spread in Central Africa in the late 1970s has shown that resistance to ampicillin, chloramphenicol and tetracycline was encoded by a single transferable plasmid of incompatibility group X [185]. These plasmids had similar restriction endonuclease digestion patterns to those found in Sh. dysenteriae strains from Somalia. When co-trimoxazole was used extensively for treatment, strains resistant to trimethoprim were found which had acquired an additional plasmid of incompatibility group I encoding resistance to trimethoprim, sulphonamide, ampicillin, chloramphenicol, streptomycin and tetracycline. This plasmid was almost certainly acquired by conjugation from the commensal faecal flora.

Probably the most significant bacterial pathogen in tropical areas, in terms of mortality, is M. tuberculosis. Since the earliest days of chemotherapy with streptomycin and para-amino salicylic acid, the development of resistance during therapy has been a major problem. We now understand the genetic basis of resistance to all of the major anti-tuberculosis agents [187]. It is interesting that they all generally arise from the selection of mutations in the genes targeted by the agents (e.g., rpoB for rifampicin; inhA for isoniazid). The development of resistance can be avoided by giving adequate doses of combinations of drugs at regular intervals. These conditions can be difficult to attain in tropical areas and, particularly when combined with high rates of HIV infection, have led to considerable problems with multi-drug resistance [187]. Antibiotic resistance in other bacteria causing respiratory infections has had a significant impact in tropical areas because of the unavailability and cost of modern agents, such as extended-spectrum cephalosporins or glycopeptides. Haemophilus influenzae, prior to the introduction of a vaccine, was the commonest cause of meningitis in the developed world and a very important cause of respiratory tract infection in adults. The association with chronic bronchitis is particularly important for tropical countries as this is a rapidly increasing condition due to rises in smoking and air pollution. High rates of resistance to ampicillin have been reported in strains collected in the developing world, with 38% of strains from eight African countries producing $\beta$-lactamase and, therefore, expressing high-level resistance to ampicillins [188]. The mechanisms can be varied around the world. In Hong Kong, despite a high rate of resistance to ampicillin (30%), only 40% of these resistant strains produced $\beta$-lactamase [189]. Resistance to agents like tetracycline is common and evidence from the UK suggests that repeated use of $\beta$-lactam antibiotics like cephalosporins results in a rise in MICs to all cephalosporins including third generation compounds [190]. The mechanism of resistance in these strains is by no means clear, but is likely to involve decreased outer-membrane permeability or decreased affinities of penicillin-binding proteins (PBPs) 3, 4 and 5, or both, although the correlation in transformation studies has not been established [191].

Streptococcus pneumoniae continues to be an important respiratory pathogen and the first outbreak of infection caused by penicillin-resistant pneumococci occurred in South Africa in 1977. These isolates were also resistant to tetracycline, erythromycin, chloramphenicol, clindamycin, streptomycin and sometimes rifampicin [192]. Resistance to erythromycin, tetracycline and aminoglycosides is generally encoded by the genes ermB, tetM and aphA3, all located on the conjugative tranposon Tn1545 [193]. Over the succeeding years a range of clones of penicillin-resistant pneumococci emerged, the mechanism of penicillin resistance being due to changed PBPs which have a lower binding affinity for benzyl penicillin. Genetic examination of these clones has demonstrated that the PBP genes consist of a 'mosaic' of long contiguous DNA sequences which are heterogeneous and derived from commensal streptococci, being incorporated by transformation and recombination [194]. The recent appearance of clones of Str. pneumoniae resistant to extended-spectrum cephalosporins in Spain and the USA raises the spectre of strains of pneumococci that are solely susceptible to glycopeptides. Interestingly, the mechanism is the same as for penicillin resistance and the genes responsible (pbp2x) represent a globally distributed gene pool shared by commensal streptococci and Str. pneumoniae [195]. Penicillin-resistant pneumococci are well distributed around the globe and, in the case of some clones, the geographical spread has been accurately mapped. Such an example is the multi-drug-resistant serotype 23F clone which probably originated in Spain and has been identified in the USA, Mexico, Portugal, France, Croatia, South Africa and South Korea [196]. Because of the lack of a consistent worldwide surveillance system it is not possible to gain a totally accurate picture of the distribution of penicillin-resistant pneumococci in tropical areas and we rely on local surveys. The situation is undoubtedly changing rapidly and results reported recently from Singapore are probably typical. In 1995, 144 strains of Str. pneumoniae were examined and 13.2% were highly resistant to penicillin (MIC $\geqslant 1.0$ mg/L) and belonged to serogroups/types 6, 9, 23, 14 and 19. Children under the age of 5 were much more likely to carry or be infected by these strains, presumably because of the increased usage of $\beta$-lactam agents in such patients [197].

Infections caused by N. gonorrhoeae and N. meningitidis are significant causes of disease in the tropical regions. In the case of N. meningitidis, resistance to penicillin due to changes in PBPs is the most frequently encountered, although a very small number of $\beta$-lactamase-producing strains have been reported [198]. Sulphonamide resistance is widespread and recently resistance to chloramphenicol has been reported [199]. This is a particularly ominous finding

for the treatment of meningitis in the developing world, as chloramphenicol is both cheap and widely used. The use of extended-spectrum cephalosporins in such a setting would be impractical, although they are used widely now in the developed world. Penicillin and tetracycline are no longer effective treatments due to high levels of resistance, particularly to penicillin, in tropical countries [200]. The resistance determinant found in plasmid-mediated tetracycline resistance in N. gonorrhoeae, TetM, has also been reported in N. meningitidis [201]. Resistance to fluoroquinolones remains rare in N. gonorrhoeae strains, but the extensive use of these compounds (usually counterfeited) in the Indian subcontinent and south-east Asia must result in increased resistance in the future. Heavy reliance is now placed on extended-spectrum cephalosporins such as ceftriaxone for treatment, particularly in tropical areas, for the more wealthy patients. There is a realistic possibility that mutations seen in the $bla_{TEM}$ gene found in Klebsiella spp. may be selected in the same gene occurring in N. gonorrhoeae, which would result in highly resistant strains, posing considerable difficulties for treatment.

The extended-spectrum $\beta$-lactamases (ESBL) conferring resistance to ceftazidime and cefotaxime found in Enterobacteriaceae have been reported from all continents of the world except Antarctica. Reports in Africa originate from North African countries, such as Algeria, Tunisia and Egypt. A recent report from South Africa identified both TEM-26 and SHV2/5 ESBLs [202]. Increased surveillance will undoubtedly reveal a substantial resistance problem caused by these $\beta$-lactamases in Enterobacteriaceae, despite relatively low usage in some countries. A preliminary report from Thailand suggests that a range of different ESBLs from a significant number of clinical isolates is present [203].

Constraints of space mean that it is not possible for this review to be in any way exhaustive. Resistance in V. cholerae and S. typhi are discussed elsewhere in this symposium report by other authors. Clearly, enteric pathogens in tropical areas are frequently resistant to antimicrobial agents and much of the literature has been reviewed [204]. Multi-drug-resistant salmonellae, other than S. typhi, are a significant problem in many tropical countries. A study from Kenya found 66% resistance to tetracycline, 26% to chloramphenicol, 16% to gentamicin, 35% to cefuroxime and 48% to ampicillin for such bacteria [205]. There is probably considerable interplay between hospital outbreaks of Salmonella spp. and the community, as was reported in Brazil [206]. The widespread usage of fluoroquinolones in tropical areas is a cause for great concern. In one study, military personnel returning from Thailand were found to carry Campylobacter jejuni resistant to ciprofloxacin in 58% of cases [207]. Recently, strains of S. typhi resistant to 4-fluoroquinolones have been reported, particularly from India and, not surprisingly, the mechanism of resistance is due to mutations in the gyrA gene [208].

The future for the successful use of antimicrobial agents in tropical areas may appear bleak, and many political and socio-economic factors are likely to make the containment of the emergence of antibiotic resistance in tropical diseases difficult. Before adequate control measures can be instituted there is an absolute need for consistent and ongoing surveillance of antibiotic resistance to be carried out. It has been argued that this is best achieved through standardised locally managed initiatives contributing to a central agency to obtain an accurate overall picture of the problem of antibiotic resistance [209]. To this end, the WHO, through the Division of Emerging and other Communicable Disease Surveillance and Control, has established the Antimicrobial Resistance Monitoring Network to reduce the import of antimicrobial resistance by improving the surveillance of resistance and promoting the correct use of antimicrobial agents. In 1998, 60% of WHO member states were participating in the submission of standardised, quantitative antimicrobial susceptibility testing data. Only by accurately defining the problem in tropical countries can a realistic attempt be made to control the problem of antimicrobial resistance.

## MULTI-DRUG-RESISTANT ENTERIC FEVER

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There are estimated to be at least 16 million cases of enteric fever annually [210] and in some areas Salmonella typhi or S. paratyphi A or B are still the most common cause of community-acquired bacteraemia [211, 212]. Resistance to the first-line and, more recently, alternative antimicrobial agents has varying geographical patterns and demonstrates the importance of continued epidemiological and microbiological surveillance in guiding therapeutic protocols. In this review, the generally accepted definition of multi-drug resistance (MDR) that will be employed is the presence of concurrent resistance to the three standard 'first-line' agents, i.e., chloramphenicol (C), ampicillin/ amoxycillin (A) and trimethoprim-sulphamethoxazole (T).

### History and geography – S. typhi

Chloramphenicol was first used in Kuala Lumpur in 1948 and chloramphenicol-resistant S. typhi was isolated in the UK within 2 years [213]. By the 1960s, similar strains had been found in several European countries and in Nigeria, and different resistance patterns were shown to be transferred between isolates by R-factors (plasmids). By 1967, S. typhi resistant to chloramphenicol, ampicillin and tetracycline had been described from Aden and Cairo

[214]. For reasons that are unclear, the African nations have otherwise generally been spared from subsequent spread of resistant strains, apart from the appearance of a few chloramphenicol-resistant strains in Natal [215]. Elsewhere, chloramphenicol resistance became widespread, with particularly large and well described outbreaks in Mexico in 1972 [216] and in Peru a few years later [217].

The spread of multi-drug resistance in India and its neighbours exemplifies the speed at which resistance can emerge, and is summarised in recent reviews [218–220]. In 1982, <15% of prevalent strains of S. typhi were chloramphenicol-resistant, but prevalent resistance patterns shifted from resistance to just C and T before 1987, to CAT in 1988 and, by 1989, 78% of isolates in Calcutta had full multi-drug resistance. Similar MDR strains were found in South India and this emergence of resistance was accompanied by a general increase in the number of cases. However, the pattern of MDR varies enormously – there was a progressive rise in the proportion of MDR strains in Bangladesh, Pakistan and Shanghai up until the early 1990s, yet <2% of isolates in Kuala Lumpur were resistant at this time. In a large subsequent epidemic of typhoid in the Philippines, multiple resistance patterns were seen but the strains isolated remained sensitive to ampicillin, and in Indonesia, prevalent strains remain fully sensitive. Overall, it is convenient to classify countries as being in zones endemic for MDR strains (e.g., most of Asia), free of MDR strains (e.g., Africa and most developed nations) or of intermediate or pseudo-endemicity, with a combination of sensitive 'local' isolates together with a significant proportion of imported MDR strains, e.g., in the Middle East [218].

Several techniques have been used to characterise MDR strains. Using antibiograms as above, it is clear that several strains of S. typhi can exist in an area concurrently. Thus, in Vellore (South India) a large outbreak of MDR S. typhi was superimposed on the background endemic pattern in the early 1990s [221]. Similarly, a number of strains of S. typhi emerged with differing resistance patterns in Rawalpindi in 1980s, to be replaced by fully MDR strains which are now becoming less prevalent again [222]. In Bangladesh, the rapid rise in MDR S. typhi in the late 1980s has started to recede in the 1990s, especially in community-based clinic isolates [223].

## Molecular basis

It has long been recognised that several phage types, especially E1 and M1, may be associated with multi-drug resistance, but others may also be found [223]. The plasmids conferring resistance are 98–120 MDa in size and are generally of the H1 incompatability group [224, 225], although plasmids of different groups have been found in Pakistan [226]. Ribotyping has also been used to discriminate between strains in Chile [227]. Such techniques are useful as epidemiological tools, and their use has confirmed that multiple strains may co-exist in an area at the same time. Thus, in Vellore, PFGE typing of DNA digests showed that at least six different clones of S. typhi were represented in the 'background' sensitive strains, but a single plasmid-associated strain was responsible for the recent epidemic of multi-resistant disease. In the same study, the genes conferring resistance were characterised as coding for chloramphenicol acetyl transferase type I, dihydrofolate reductase type VII and TEM-1 $\beta$-lactamase [228]. It will be interesting to see if the same iso-enzymes are incriminated in MDR strains from other areas.

## S. paratyphi

Until recently, there have been only a few sporadic reports of mixed resistance patterns in S. paratyphi. However, epidemics of S. paratyphi A in India [229], Thailand and Pakistan have been reported recently. In Pakistan, the epidemic strain(s), which are fully resistant with high MICs of the three first-line drugs, have now prevailed in Rawalpindi for several years, although the associated plasmids have not yet been characterised [230]. Occasional S. paratyphi isolates have also been reported to show independent fluoroquinolone resistance [231].

## Fluoroquinolone resistance

Fluoroquinolones have become the treatment of choice for enteric fever in Asia over the past decade. Unfortunately, their introduction was rapidly followed by the emergence of resistance, which has now become a major problem. Resistance is chromosomally mediated, with a number of different mutations in the gyrA gene already reported [232, 233], and is independent of plasmid-mediated resistance to other antibiotics. The initial findings in South India were of a gradual rise in MICs [234] and similar observations were made in Vietnam [235]. However, although the latter strains were still sensitive to ofloxacin by conventional criteria, the small increase in MIC was clinically significant, especially if disk sensitivity tests were used to detect nalidixic acid resistance as a simple laboratory marker [235, 236]. Clinical failures of short-course ofloxacin treatment were clearly linked to nalidixic acid resistance, with three of five patients with nalidixic acid-resistant strains failing therapy compared with 4 of 90 with sensitive isolates [236].

Fluoroquinolone resistance was initially perceived to be a problem only in areas such as Vietnam where short-course chemotherapy was being studied as a possible option for overcoming costs and logistic difficulties in treating patients. However, the high prevalence of fluoroquinolone resistance threatened such an approach in the large recent epidemic in Tadzikistan [237] and significant resistance is now seen in over a third of

isolates imported to the UK [238], emphasising the rapid spread of such resistance around the Indian subcontinent – the source of most imported British cases.

## Clinical implications

Laboratory tests of drug resistance are not always clinically relevant – thus, many laboratories have traditionally included tests for aminoglycoside and tetracycline resistance in Salmonella spp., although these agents have little clinical role in treating enteric fever. Despite the sensitivity of isolates to aztreonam in vitro, this agent was not useful clinically in Peru [239]. Unfortunately, resistance in vitro to chloramphenicol, ampicillin, trimethoprim-sulphamethoxazole and fluoroquinolones is reflected in treatment failures. It is still not clear whether MDR strains are also inherently more pathogenic. Earlier reviews suggested worsened outcomes in children infected with MDR strains, but much of this could be explained by the late presentation of patients to tertiary care facilities after inadequate treatment with ineffective first-line therapies [218, 240]. Nevertheless, infection with resistant strains is associated with higher quantitative levels of bacteraemia in Vietnam, suggesting an intrinsic increase in the pathogenicity of MDR strains [241].

In geographical areas where resistance is uncommon (i.e., Africa) and facilities for blood culture and antimicrobial sensitivity tests are not available, traditional empirical therapy with chloramphenicol or its alternatives is still an acceptable approach. In other areas, new strategies have evolved, principally the use of second generation or later cephalosporins or fluoroquinolones, as reviewed recently [231, 242]. Ceftriaxone is well-established and cefixime is a promising agent, although cephalosporins generally take longer to clear fever than fluoroquinolones and are prohibitively expensive for routine use in resource-poor countries. There was little to choose between the earlier fluoroquinolones, and as resistance to these has become widespread, independent resistance to cephalosporins has recently been reported in Bangladesh [243], so new therapeutic approaches are needed. Initial promising reports of results with a cheap oral nitrofuran (furazolidone) in India need to be confirmed [244]. Early studies in Egypt with azithromycin were disappointing, but recent experience has been more encouraging [245], and this agent may become the mainstay of our approach for the near future, possibly in combination with other drugs.

## Summary

In summary, MDR strains of S. typhi are both epidemic and endemic in many countries in Asia and MDR S. paratyphi A has recently emerged in Pakistan. Multiple clones may be present in a given area at any time. Fluoroquinolones and third generation cephalosporins have been used widely over the past decade to treat MDR strains. The clinical superiority of fluoroquinolones is now threatened by the rapid emergence of chromosomally mediated resistance and cephalosporin resistance is also being reported. Whether these problems can be overcome by the use of newer fluoroquinolones or cephalosporins remains to be seen. Meanwhile, furazolidone and azithromycin deserve further trials, and clinical and molecular surveillance of resistance patterns remains essential.

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