

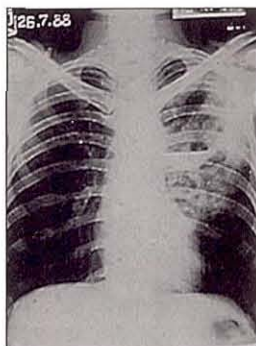
The CAMP test for Listeria. Enhanced haemolysis is shown by –
Top: *Listeria monocytogenes*
Centre: *Listeria ivanovii*

Melioidosis

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A possibly under-diagnosed but important cause of severe infection in the tropics.

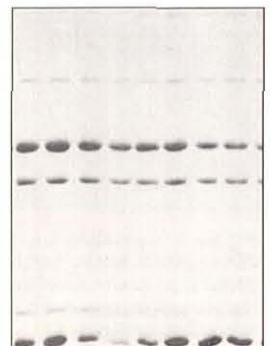


Iron and Micro-organisms

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As almost all micro-organisms need iron, they have had to develop special strategies to acquire it.



Melioidosis

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Introduction

The name melioidosis describes any infection of humans or animals caused by the saprophytic Gram-negative bacillus *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*). In recent years it has emerged as an important cause of severe community-acquired infections in south east Asia and northern Australia, although asymptomatic infections are more common. The incidence of the disease had previously been under-recognised, and it is possible that it remains under-diagnosed in many other areas of the tropics. Although rarely imported to temperate climates, melioidosis should be considered in the differential diagnosis of any febrile illness in someone who has ever lived in an endemic area, since it has a remarkable propensity for latency, giving rise to the nickname 'Vietnam time bomb'.

Clinical manifestations

The clinical presentation of melioidosis is extremely variable, and one form of the disease may progress to another, making classification difficult. Most infections are asymptomatic and manifested only by seroconversion. Up to 80% of children in north east Thailand have low level antibodies by the time they are 4 years old, suggesting that exposure is almost universal.¹ On the other hand, the most common clinical presentation of the dis-



Figure 2: Suppurative parotitis with abscess formation is a common manifestation of melioidosis in children. (Reproduced from Dance DAB. *Melioidosis, glanders and tularaemia*. 1992. *Medicine International* 107: 4510 by kind permission of The Medicine Group (Journals) Ltd.)

ease (60% in Thailand) is a severe, rapidly progressive septicaemia with a high mortality rate.² In between these extremes, *B. pseudomallei* may cause infections which are acute or chronic, localised or disseminated. It is a pyogenic organism, which produces abscesses or granulomata depending on the duration of the infection. Septicaemic patients frequently have abscesses scattered throughout the body, particularly in the lungs, liver, spleen, skin and soft tissues. Localised disease may also occur in any organ, but is commonest in the lung, mimicking tuberculosis by causing cavitation (Figure 1). In children, a frequent manifestation is suppurative parotitis leading to parotid abscesses³ (Figure 2).

Geographical distribution

Melioidosis was first recognised in Burma in 1911, and was subsequently described in many other countries in south east Asia, notably Malaysia, Vietnam, Cambodia and Indonesia. During the last two decades, more cases have been reported from Thailand than any other country, particularly from the north east (Khon Kaen and Ubon Ratchatani). Since 1950, a focus of the disease has been recognised in northern Australia (far north Queensland and Northern Territory), and increasing numbers of cases have been diagnosed there in recent years. Sporadic cases have also been associated with Papua New Guinea, the Philippines, Pacific Islands, Hong Kong and mainland China, and a

surprisingly large number of cases have been identified in Singapore over the past five years. Elsewhere, the picture is less clear. *B. pseudomallei* has been isolated occasionally from human or animal infections, or the environment, in the Indian subcontinent, Turkey and Iran, several countries in central Africa, central and South America, and the Caribbean. The true incidence of melioidosis in these areas is unknown, since awareness is low amongst clinicians and laboratory workers, and the rural populations exposed rarely have access to laboratories capable of making the diagnosis. Although cases are occasionally imported into temperate countries, the only known example of transmission in such an environment was an outbreak which occurred amongst horses and zoo animals in France in the mid 1970s.⁴ The distribution of the disease was comprehensively reviewed in 1991.⁵

Host range

B. pseudomallei has one of the broadest host ranges of any bacterial pathogen. Infection has been recorded in sub-human primates, rats, mice, rabbits, guinea pigs, cats, dogs, cattle, sheep, goats, pigs, horses, deer, camels, koala bears, kangaroos and a panda. Furthermore, a handful of cases have been reported in birds (galah, cockatoo, pigeon), which have a high body temperature, and cold blooded creatures such as a crocodile. Even dolphins and killer whales have been infected by *B. pseudomallei*.

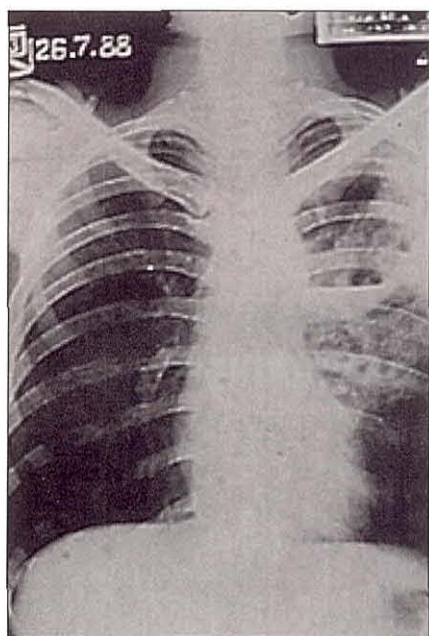


Figure 1: Cavitating lesion in the left upper zone surrounded by consolidation—a typical picture of pulmonary melioidosis. (Reproduced with permission from the Editor, PHLS Microbiology Digest.)



Figure 3: The natural habitat of *B. pseudomallei*. Infection is thought usually to be acquired by inoculation in those working barefoot in rice paddy. (Copyright Dr A.E. Brown).

Epidemiology

Although melioidosis was long considered to be a zoonosis transmitted by rodent excreta, French workers in Vietnam showed that, in reality, *B. pseudomallei* was an environmental saprophyte, and that both man and animals were victims of exposure to the organism in soil and muddy water. It is particularly found in rice paddy⁶ (Figure 3). Infection is thought, usually, to result from inoculation or contamination of wounds or mucosal surfaces, although a specific episode of exposure is rarely identified.⁷ Inhalation of aerosols probably accounted for the disproportionate number of cases in helicopter crew during the Vietnam war⁸ and for two laboratory-acquired infections. The role of ingestion is unclear. Other, rare modes of acquisition include iatrogenic inoculation and person-to-person spread.

Melioidosis is thus mainly acquired by people in regular contact with soil and water, such as rice farmers in south east Asia and aboriginals in Australia. Several hundred cases are reported annually in Thailand, and the incidence in one province has been estimated as 4.4/100,000 per annum.⁷ In this study, there was a bimodal age distribution, with the main peak occurring in the 40–60 year age group, and the male:female ratio was 3:2.⁷ The occurrence of disease is markedly seasonal, with a peak occurring during the rainy season, suggesting that most cases are recently acquired. In north east Thailand, *B. pseudomallei* accounts for almost 20% of community-acquired septicaemia and out-numbers both *Staphylococcus aureus* and *Escherichia coli* in blood cultures during the rainy season.² However, the disease may only appear after long latent periods, the record being 26 years after leaving an endemic area.⁹ The proportion of seropositive persons who are latently infected is unknown.

Pathogenesis and virulence

The outcome of contact with *B. pseudomallei* depends on the balance between host immunity and the virulence and size of

the inoculum. More than 70% of patients with acute, severe melioidosis in Thailand are immunocompromised in some way, particularly by diabetes mellitus or chronic renal disease.² Other associated conditions include malignancy, immunosuppressive treatment (particularly steroids), liver disease, other chronic conditions, alcohol or drug abuse, and pregnancy. Little is known about the specific immunological mechanisms responsible for protection, although cell-mediated immunity is probably important. Relapses also usually occur at times of intercurrent stress (e.g. trauma or burns, onset of malignancy or diabetes, other acute infections), so it is perhaps surprising that melioidosis has not appeared to increase in association with the spread of human immunodeficiency virus infection in Thailand.

Although strains of *B. pseudomallei* appear to differ in virulence for experimental animals, virulence factors of the

organism are poorly understood. It possesses lipopolysaccharide endotoxin which presumably contributes to the pathogenesis of septicaemic disease, although a heat-labile exotoxin may also be involved in lethality.¹⁰ Host-derived cytokines (tumour necrosis factor, interleukins) probably act as the final common pathway during septicaemia.¹¹ Haemolysins, proteases, lecithinase and lipase may contribute to local tissue damage. Intracellular survival and the formation of abscesses presumably contribute to the refractory and persistent nature of melioidosis.

Diagnosis

B. pseudomallei is an ovoid, oxidase positive, motile Gram-negative bacillus. It has been classified in various genera (*Pleifferella*, *Malleomyces*, *Loefflerella* etc.) in the past, and until recently it was included with *P. mallei*, *P. pickettii* and *P. cepacia* in RNA homology group II of the genus *Pseudomonas*. Recent work by Yabuuchi *et al.* has supported the transfer of this group to a separate genus, named *Burkholderia* after the American bacteriologist WH Burkholder.¹²

Since melioidosis is difficult to diagnose on clinical grounds alone, specific diagnosis depends on the detection of *B. pseudomallei* or of corresponding antibodies. A Gram stain of pus or secretions may reveal bipolar or unevenly staining Gram-negative rods (Figure 4), although this appearance is not reliable. Immunofluorescence is more specific,¹³ and other rapid diagnostic techniques are under development. Isolation and identification of *B. pseudomallei* is relatively easy. It grows well on most culture media, forming rugose or smooth colonies after 48 hours (Figure 5) and producing a sweet, earthy smell. The isolation rate from sites with a normal flora may be increased using selective techniques. The solid medium described by Ashdown¹⁴ has proved most useful, and various selective enrichment broths have also been employed with success. The organism has often been misidentified as a

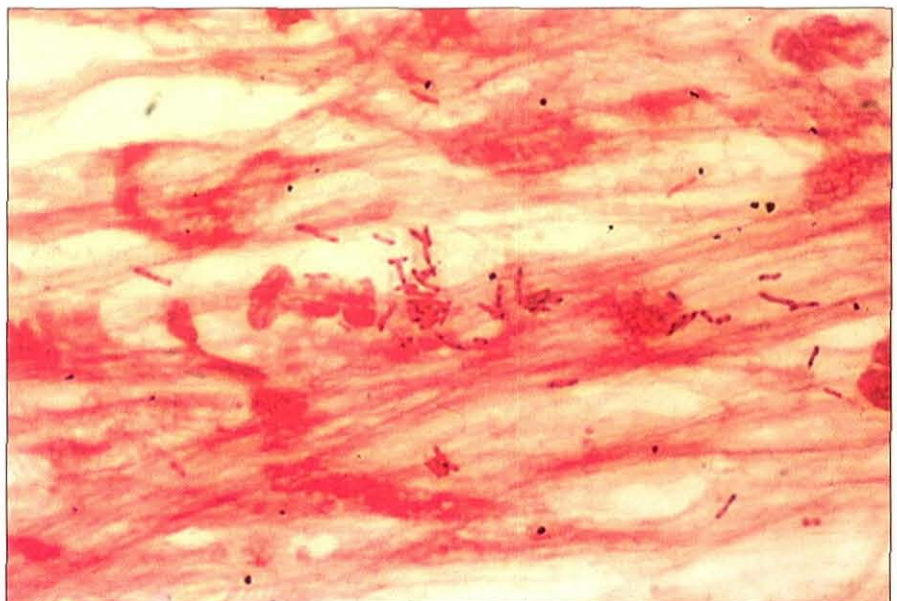


Figure 4: Gram stained smear of sputum from a patient with melioidosis. Unevenly staining Gram-negative bacilli are seen, which may exhibit 'safety pin' bipolarity. (Copyright Dr MD Smith.)

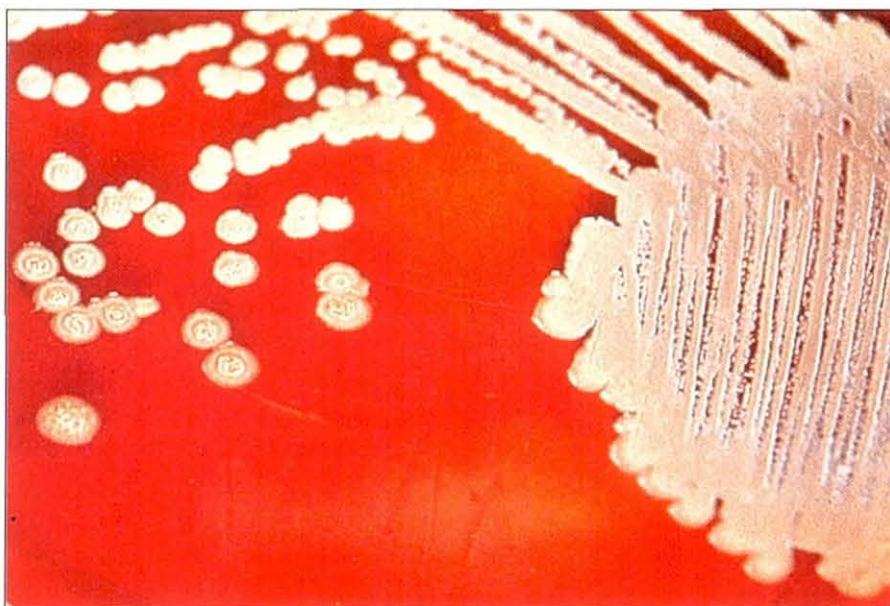


Figure 5: *B. pseudomallei* colonies on blood agar after 48 hours incubation. These may be discarded as contaminants by the unwary, because of a resemblance to colonies of *Bacillus* species. (Copyright Dr DAB Dance.)

contaminant by workers unfamiliar with its appearance, but is readily identified by modern biochemical test kits such as the API 20NE.¹⁵ It can be distinguished from *P. aeruginosa* by absence of pigmentation in most strains, failure to grow in cetrimide-containing media, and a characteristic antibiotic susceptibility pattern (see below). It must be distinguished from *P. cepacia*, with which it shares many features, by serological (slide agglutination immunofluorescence), biochemical (arginine dihydrolase, lysine decarboxylase, ONPG, nitrate reduction to gas) or antibiotic susceptibility (co-amoxiclav) tests. **This is a dangerous organism, listed in Hazard Group 3, therefore, appropriate safety precautions should be taken. Suspected cultures should be referred to a Reference Laboratory for confirmation.**

Various tests have been described to detect antibodies to *B. pseudomallei*. The most widely used is an indirect haemagglutination (IHA) test, which employs heat-stable antigens (probably predom-

antly lipopolysaccharide). This is useful in patients from non-endemic areas, in whom a single IHA titre of > 1:40 at presentation is highly suggestive of melioidosis. However it has a low specificity for diagnosis of acute melioidosis in people continually exposed to *B. pseudomallei*. ELISAs which detect IgG give similar results, whilst assays which detect specific IgM (indirect immunofluorescence, ELISA) correlate better with disease activity.¹⁶

Susceptibility, treatment and prevention

B. pseudomallei may be tested for susceptibility to antibiotics using conventional methods (disk diffusion, agar or broth dilution). Interpretation is straightforward with the exception of trimethoprim and sulphonamides (singly or in combination), which give very indistinct end points irrespective of methodology. The organism is intrinsically resistant to aminoglycosides, polymyxins, early penicillins and cephalosporins, and is relatively resistant to the fluoroquinolones.¹⁷ It is susceptible to

chloramphenicol, tetracyclines and some new beta-lactams such as ceftazidime, imipenem, and co-amoxiclav. Treatment with these latter antibiotics, along with aggressive supportive therapy, has reduced the mortality, although this remains high (approximately 40–50%) in severe disease.¹⁸ Antibiotics are usually given for at least two weeks parenterally, followed by between 6 weeks and 6 months of oral treatment, but the relapse rate is still over 20%.¹⁹ Resistance to antibiotics can emerge during treatment in 5–10% of cases.^{17,19}

Experimental vaccines have been evaluated in animals with varying results, but none has been used in humans. The only practical preventive measure is avoidance of contact with *B. pseudomallei* in the environment, hospital or laboratory, particularly by 'at-risk' individuals such as diabetics, although this is very difficult for rice farmers in endemic areas.

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Iron and Micro-organisms

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Introduction

Almost all living cells, whether microbial, plant or animal, may require iron as an essential element. Iron is needed for important cellular functions, such as biochemical oxidation-reduction reactions. Since iron has an extremely wide redox potential, it is a very versatile biocatalyst. A variety of enzymes that require iron for activity have been characterised; ribotide reductase, nitrogenase, peroxidase, catalase, cytochromes and others. These enzymes function in the reduction of ribonucleotides, dinitrogen and the activation or decomposition of peroxides. Cells need to limit levels of available free iron to avoid production of toxic hydroxide radicals by the Haber-Weiss-Fenton reaction.

Among all elements on the surface of planet Earth, iron ranks fourth in abundance among the metals. Ferrous (iron II) iron is quite soluble and one can obtain a

100mM solution at pH 7. The solubility product constant of ferric iron (iron III) may be as small as 10^{-38} M, which would limit the amount of free ferric iron that can be dissolved in water at pH 7.4 to about 10^{-18} M.

Most iron in mammalian hosts is found intracellularly in ferritin, haemosiderin, and haemoproteins. Extracellular Fe^{3+} is bound to lactoferrin (LF) in exocrine secretions, such as saliva, tears, and mucosal secretions, and also to transferrin (TF) in plasma. One consequence of extracellular iron sequestration is that LF and TF bind Fe^{3+} with such high avidity that many micro-organisms cannot remove the iron for microbial metabolism.

Micro-organisms need approx. 0.4–4.0 μ M iron. Because of the insolubility of iron and iron sequestration by lactoferrin or transferrin, micro-organisms have evolved a number of mechanisms for the acquisition

of adequate iron from the environment.

This paper focuses on the iron acquisition strategies of pathogenic bacteria, fungi and yeasts as well as some practical applications of them.

Pathogenic bacteria

Micro-organisms grow under differing conditions of iron limitation. It can therefore be assumed that they developed different iron acquisition systems to meet these various iron conditions. Almost all pathogenic bacteria exhibit more than one strategy to overcome iron limitation. The different mechanisms to obtain sufficient quantities of iron to support their metabolism and growth are summarised in **Table 1**.

Utilisation of host iron compounds

A number of pathogenic bacteria are able to utilise iron bound to haem or haemo-

Table 1: Summary of mechanisms of iron uptake by pathogenic bacteria. (Modified from Wooldridge and Williams.¹)

Species	Endogenous siderophores	Exogenous siderophores	Host iron compounds	Others
<i>Acinetobacter baumannii</i> <i>Aeromonas hydrophila</i> <i>Bordetella pertussis</i>	catecholate-type siderophore ¹⁸ Amonabactins	ferrioxamines ferrioxamines	Transferrin, Ovotransferrin, Lactoferrin	reductases
<i>Brucella abortus</i> <i>Campylobacter</i> spp. <i>Citrobacter</i> spp. <i>Enterobacter</i> spp. <i>Escherichia coli</i>	2,3-dihydroxybenzoic acid ¹⁹ Enterobactin, Aerobactin Enterobactin, Aerobactin Enterobactin, 2,3-dihydroxybenzoic acid, linear 2,3-dihydroxybenzoylserines, Aerobactin	Enterobactin ferrioxamines ferrioxamines Ferrichrome, Ferricrysin, Ferricrosin, Coprogen, Rhodotorulic acid, citrate, (Ferrioxamine B)	Haem, Haemoglobin Haem	α -Haemolysin, Ferrous iron
<i>Haemophilus influenzae</i>		Enterobactin	Haem, Haemoglobin, Transferrin	
<i>Klebsiella pneumoniae</i>	Aerobactin, Enterobactin, Ferrioxamine-type	Aerobactin, ferrioxamines		
<i>Legionella pneumophila</i> <i>Listeria monocytogenes</i>		ferrioxamines, citrate Ferrichrome	Transferrin ²⁰	reductases ⁷ reductase ⁸
<i>Mycobacterium smegmatis</i> <i>Neisseria meningitidis</i> / <i>N. gonorrhoeae</i> <i>Pasteurella multocida</i> / <i>P. haemolytica</i> <i>Proteus</i> spp.	Exochelin, Mycobactin	Aerobactin, Schizokinen, Arthrobactin Ferrioxamines, Rhizoferrin	Haem, Haemoglobin, Transferrin, Lactoferrin Transferrin	
<i>Pseudomonas aeruginosa</i>	Aerobactin, α -keto- α -hydroxyacids Pyoverdins, Pyochelin	Enterobactin		
<i>Pseudomonas cepacia</i> <i>Salmonella</i> spp.	Ornibactins ²¹ Enterobactin, Aerobactin 2,3-dihydroxybenzoic acid, linear 2,3-dihydroxybenzoyl-serines α -keto- α -hydroxyacids Aerobactin, Enterobactin	Enterobactin, Ferrichrome, ferrioxamines ferrioxamines, Ferrichrome, Ferricrysin		
<i>Serratia</i> spp.		ferrioxamines		
<i>Shigella</i> spp. <i>Staphylococcus aureus</i>	Aerobactin, Enterobactin Staphyloferrin A, B	ferrioxamines	Haem Haem, Haemoglobin	
<i>Streptococcus mutans</i> <i>Vibrio anguillarum</i> <i>Vibrio cholerae</i> <i>Yersinia enterocolitica</i>	Anguibactin, Enterobactin-like Vibriobactin Yersiniabactin, ²²	Aerobactin, ferrioxamines	Haem, Haemoglobin Haem, Haemoglobin	reductase Haemolysin

With acknowledgement to Jonas Aki

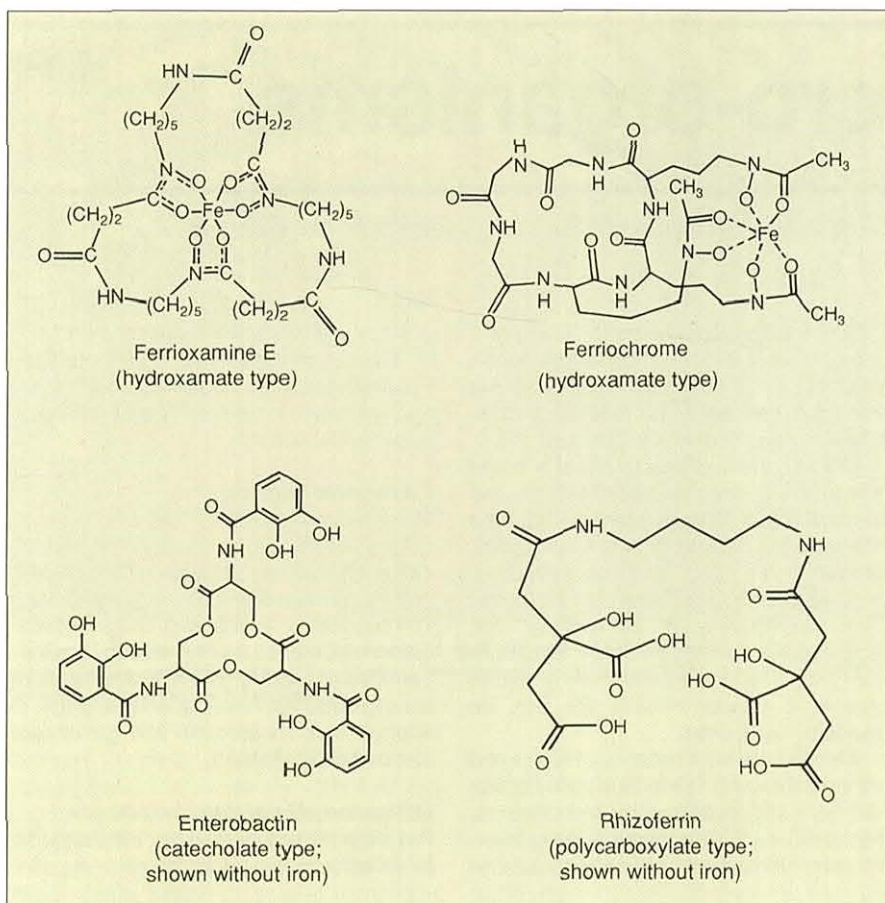


Figure 1: Chemical structures of typical siderophores.

globin. *Escherichia coli* and *Yersinia* spp. utilise haem as an iron source, whilst *Neisseria meningitidis*, *N. gonorrhoeae*, *Haemophilus influenzae*, *Vibrio cholerae* and *Campylobacter jejunii* use both haem and haemoglobin. The mechanisms by which iron (or haem) is acquired from these complexes is not understood, although it is known that the process does not require siderophores. Genes, encoding outer membrane proteins which are required for uptake from haem, have been cloned from *Y. enterocolitica* and *E. coli*. *Haemophilus influenzae* has an absolute requirement for

porphyrins, which if supplied as haem (ferritroporphyrin IX), can be used as a source of both iron and porphyrin. It has been suggested that levels of free haem in normal serum are too low to support the growth of pathogens. Some microorganisms however, are able to increase the availability of free haemoglobin (and thus haem) in blood by secreting hemolysins.

Some pathogenic bacteria extract iron directly from transferrin or lactoferrin. *Neisseria gonorrhoeae* and *N. meningitidis* scavenge sufficient iron from humans by this means. Iron starvation of both *N.*

meningitidis and *N. gonorrhoeae* results in the production of several iron-repressible outer membrane proteins which could conceivably function in the acquisition of iron from both glycoproteins.² Such receptors were detected in a number of different bacteria capable of acquiring iron from these compounds.

Uptake of iron by siderophores

One of the strategies employed by microorganisms to obtain iron involves production and uptake of siderophores. Siderophores are low molecular weight (0.4 to 2 kDa) iron chelating agents, synthesised in response to iron stress. These substances bind iron (III) and facilitate its transport into the cell. Siderophores exhibit highly complex formation constants for ferric iron. The chelating groups are catecholates, hydroxamates, polyhydroxycarboxylates and α -keto- α -hydroxy acids. Typical members of each siderophore-structure are shown in Figure 1. Siderophores in pMol amounts effect iron supply.

Growth of bacteria in iron-limiting nutrient media induces the synthesis of iron-regulated outer membrane proteins (IROMPs); furthermore, changes in the composition of other outer membrane proteins were also seen in SDS-PAGE (Figure 2). Such IROMPs act as receptor proteins for ferric siderophores. Under iron-limiting conditions *E. coli* induced the synthesis of at least seven IROMPs. Some of them are simultaneously receptors for phages and colicins. The specificity of these IROMPs is shown in Table 2.

The genes of *E. coli*, and other Gram-negative bacteria involved in iron uptake are known to be negatively regulated by the *fur* repressor protein.³

Iron uptake employed by the siderophores-system involves the following steps:⁴

1. Binding of the ferrisiderophore to the outer membrane via IROMP;
2. Transfer of the ferrisiderophore from its IROMP-complex to a carrier protein. This step is dependent on *tonB* encoding a protein involved in energy-coupled transport-processes across the outer

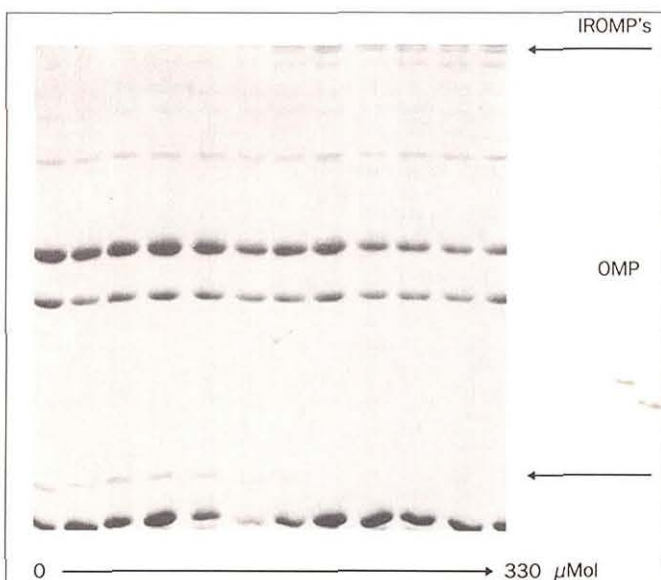


Figure 2: Polyacrylamide gel electrophoresis of *E. coli* AB 2847 (*aroA*), cultivated in Tryptic soy broth containing increasing amounts of the artificial iron-chelator α, α' -bipyridyl (μ Mol) to obtain iron limited conditions.

Table 2: Siderophore-uptake systems in <i>E. coli</i> .		
IROMP	Molecular size [kDa]	Fe (III) siderophore bound
<i>FepA</i>	81	enterochelin (synonymous with enterobactin)
<i>FhuA</i>	78	ferrichrome (produced by fungi)
<i>FhuE</i>	76	coprogen (produced by fungi)
<i>FecA</i>	80.5	dicitrate
<i>Cir</i>	74	Monocatecholate, 2,3-dihydroxybenzoic acid as the precursor of enterobactin; 2,3-dihydroxybenzoylserines as breakdown products of enterobactin
<i>Fiu</i>	83	
<i>IutA</i>	74	Aerobactin

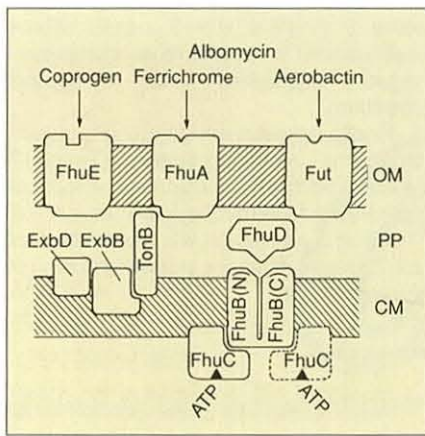


Figure 3: Model of iron(III) hydroxamate transport. OM, outer membrane; PP, periplasm; CM, cytoplasmic membrane. (From W Köster (1991). *Biol. Metal.* 4: 23–32, by permission.)

membrane and by the ExbB/ExbD proteins (see **Figure 3**).

3. Release of the ferrisiderophore from its complex with the periplasmic carrier protein to a cytoplasmic membrane protein complex, which, in an energy-dependent process, translocates the ferrisiderophore across the cytoplasmic membrane;
4. Release of the ferrisiderophore complex into the cell where the iron will be released, most likely by a reductive mechanism.

Almost all bacteria exhibit a number of IROMPs including the uptake systems of self-produced (endogenous) siderophores or those produced by other micro-organisms (exogenous siderophores). Micro-organisms in mixed cultures can cross-feed siderophores produced by each of them.

A mechanistically novel iron-III-transport system was detected in *Serratia marcescens*, so called *sfuABC*.⁵ The *sfuABC* genes encoded three proteins in the cell wall suggesting a periplasmic-binding-protein-dependent iron transport mechanism. This *sfuABC* system acts very effectively in the utilisation of α -keto- α -hydroxy acid mediated iron uptake, e.g. in *E. coli* and *Salmonella* spp.

Reduction of ferric iron and transport of ferrous iron

Most bacterial species that produce siderophores possess a ferric reductase specific for that particular iron chelator. Although some of these ferrisiderophore reductases have marginal activity with ferric citrate, reductases specific for ferric citrate have been identified in several bacterial species. Mostly such reductase activities are located in the cell wall extracts. Some bacteria excrete soluble ferri-reductases on the surface of the cell wall to reduce ferric iron. Simultaneously, these ferri-reductases function also as sulphite-reductase. Ferri-reductases are responsible for blackening (FeS) colonies of bacteria on selective nutrient agar.

The only known bacterial reductase specific for transferrin or lactoferrin was found in *Listeria monocytogenes*.⁶ Two ferri-reductases were detected in *Legionella pneumophila*, a bacterium unable to produce siderophores.⁷

Much less is known about the uptake of ferrous iron, although there are some micro-organisms which mainly or exclusively use ferrous iron for their iron supply, e.g. *Bifidobacterium bifidum*, *Streptococcus mutans*. *Escherichia coli* has an iron (II) transport system (*feo*) which may make an important contribution to the iron supply of the cell under anaerobic conditions. The iron (II) transport genes encode for two proteins (*feoA* and *feoB*) localised in the cytoplasmic membrane.⁸

Role of iron regulation of virulence genes

Since free iron is extremely limited in the mammalian host, a shift from a high to a low-iron environment is an important environmental signal to bacteria to co-ordinate the regulation of gene expression. Iron serves as a central regulatory substance which controls the expression of almost 40 genes in *E. coli*. Besides the iron uptake systems which were induced, other virulence factors are known to be under the control of iron⁹ (**Table 3**).

Iron storage proteins

When iron availability is in excess of the required level, it is potentially toxic to all cells. Ferritin has evolved for the sequestration of iron in a safe, but bioavailable form. Bacterioferritin, a multimeric iron-storing haemoprotein, resembles the ferritins in animals and plants although distantly related in evolution from other ferritins, and may perform an analogous function in iron detoxification and storage. Most analyses have been carried out on the bacterioferritins of *E. coli*, *P. aeruginosa*, and *Azotobacter vinelandii*. Their presence was first suspected from cells grown in iron-enriched media.¹⁰ *Helicobacter pylori*, occurring in the stomach, is supplied by an excess of iron based on availability at low pH. *Helicobacter pylori* produces an iron-containing protein resembling ferritin which could have a major role in storing any excess of iron of this bacterium.¹¹

Fungi and yeasts

The strong competition for iron between saprophytic fungi and bacteria on the one hand, and microbial parasites and hosts on the other, may be responsible for siderophore production. Most fungi excrete di- and tri-hydroxamate type siderophores, although *Mucor*, *Phycomyces*, *Rhizopus*, and many other fungi seem to be unable to produce hydroxamate siderophores. More-

over, several ascomycetous and basidiomycetous yeasts, such as *Saccharomyces*, *Lipomyces*, *Geotrichum*, *Candida* and *Cryptococcus*, have never been shown to produce siderophores.

Ornithine is an essential part of all fungal siderophores (ferrichromes, asperchromes, coprogens, fusigens, fusarines, rhodotorulic acid) produced by Ascomycetes, Basidiomycetes and Deuteromycetes. Zygomycetes produce polycarboxylate-type siderophores, e.g. rhizoferrin from *Rhizopus microsporus* var. *rhizopodiformis*, which is known as an agent of Mucormycosis.

Siderophores are also taken up by fungi after being complexed with iron. The conformation of ferrisiderophores is crucial for recognition and transport by the membrane located transport systems. e.g. uptake of ferrichrome in *Neurospora crassa* is highly stereospecific.

After the iron is removed from the ligand inside the cell, the free ligand reappears in the medium and may serve for another round of iron transport ('shuttle mechanism'). Hydroxamate siderophores act in some fungi also as storage compounds of iron, as well as ferritin in the route of rhizoferrin.¹²

It is well established that iron is also an essential element for the growth of yeasts. The oxidation state of iron is a determinant of iron uptake by *Saccharomyces cerevisiae*. Fe(II) uptake depends upon a transplasma membrane redox system, which is induced in iron-deficient conditions.¹³

Candida albicans is able to recognise a variety of hydroxamate siderophores; *Cryptococcus neoformans* has also been shown to respond to exogenously-supplied ferrioxamine B¹². Adherence of *Candida albicans* to epithelial cells and its propensity for germ tube formation was shown to be under the control of iron. Differences in yeast cell wall showed quantitative but no qualitative differences in the protein profile of iron-rich and iron-deplete organisms. These results indicate that expression of important virulence attributes by *Candida albicans* is highly dependent on available iron and that expression *in vivo* may therefore be significantly different from that observed under conventional laboratory conditions.¹⁴

Application of siderophores in practice

Naturally occurring and synthetic ferric iron chelators are currently of great interest because of their role in micro-organisms

Table 3: Examples of iron-regulated bacterial virulence determinants.

Organism	Virulence determinants
<i>E. coli</i>	shiga like toxin I (SLT-I)
<i>Shigella dysenteriae</i> Type 1	shiga toxin
<i>Serratia marcescens</i>	Hemolysin
<i>Pseudomonas aeruginosa</i>	exotoxin A elastase alkaline phosphatase
<i>Corynebacterium diphtheriae</i>	Diphtheriae toxin

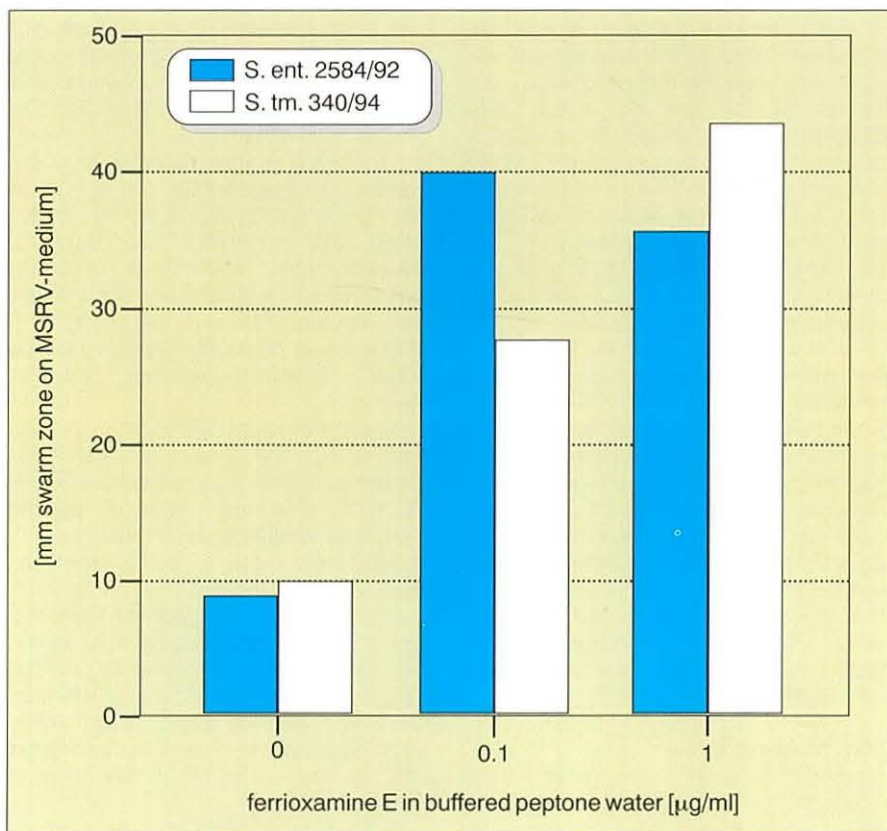


Figure 4: Effectiveness of ferrioxamine E supplementation of buffered peptone water on motility of *Salmonella* strains on MSR/V-medium.

and plants; they have potential applications in the treatment of iron overload diseases, as a part of siderophore antibiotics, as biomimetic iron carriers for use in malaria therapy and recently as selective growth factors in microbial culture. High pH and the presence of ovotransferrin in albumen causes an iron limitation for *Salmonella* spp. Addition of ferrioxamine E to buffered peptone water, as used in the ISO methods for *Salmonella* spp. isolation, functions as a selective growth factor to overcome this iron limitation. A significant increase in sensitivity and a shorter incubation time for the isolation of *Salmonella* spp. was observed for all serotypes

tested.¹⁵ Growth and motility of *Salmonella* spp. on MSR/V-medium was extensively influenced by supplementation of buffered peptone water with ferrioxamine E (Figure 4).

Research groups are currently working on the development of siderophore antibiotic conjugates. Increased activity of such compounds containing a siderophore moiety e.g. of catecholate type, linked to a drug, smuggle the antibiotic into the cell via siderophore transport systems and act as 'Trojan horses'.¹⁶

Susceptibility testing to desferrioxamine B by an agar diffusion method can be used as a diagnostic tool differentiating all *Staphylococcus epidermidis* as well as

some *S. hominis* and *S. capitis* strains (susceptible) from the other coagulase-negative staphylococci and *S. aureus* (resistant).

Finally, an overview on iron and micro-organisms should mention Desferal® (desferrioxamine B) which is biotechnologically produced by *Streptomyces pylosus*. This drug is very effective in the treatment of iron overload diseases and was the drug of the year in 1991.

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