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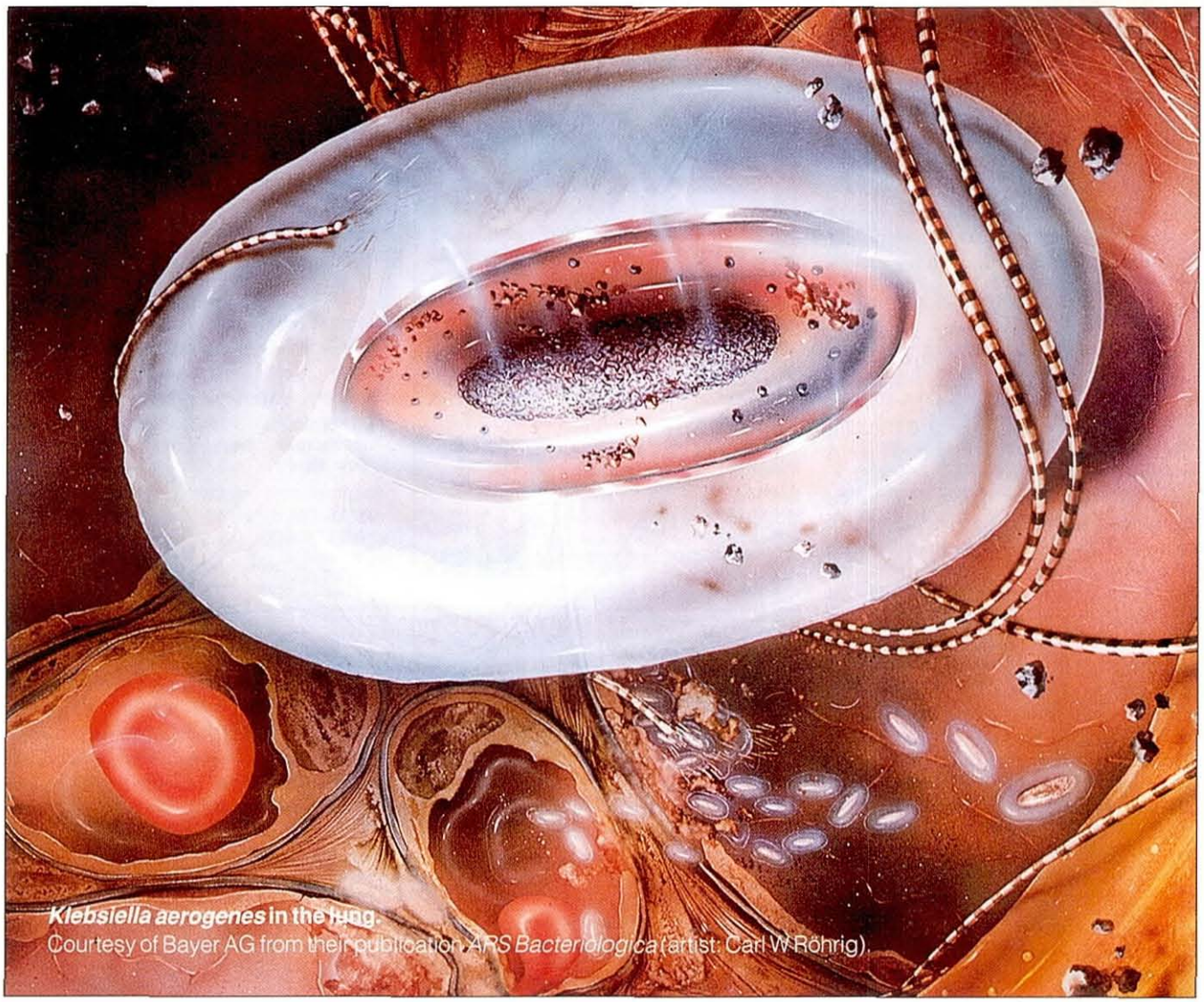
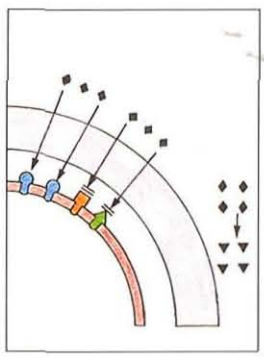


***Klebsiella aerogenes* in the lung.**
Courtesy of Bayer AG from their publication *ARS Bacteriologica* (artist: Carl W Röhrig).

Methicillin-resistant staphylococci-evolution and expression of resistance

Peter E Reynolds,
University Lecturer,
Department of Biochemistry,
University of Cambridge,
Cambridge, UK.

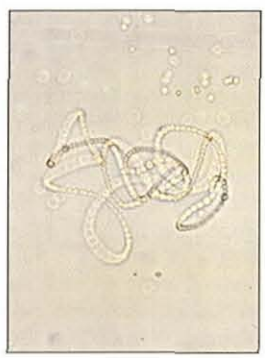
Nosocomial resistant staphylococci may reach either 20% (MRSA) or 50% (MRSE) of strains isolated.



The relevance of cyanobacteria (blue-green algae) to public health

Paul R Hunter,
Director & Consultant
Medical Microbiologist,
Chester Public Health
Laboratory, Chester, UK.

There is a great wealth of experimental evidence that cyanobacterial toxins can cause severe adverse health effects.



Methicillin-resistant staphylococci-evolution and expression of resistance

Peter E Reynolds, MA, PhD, University Lecturer, Department of Biochemistry, University of Cambridge, Cambridge, UK.

Introduction

The abbreviations MRSA and MRSE were used originally to describe methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*: in the medical literature they continue to be used predominantly for this purpose. However, several of these isolates have acquired resistance to other antibiotics as well as heavy metals and some disinfectants; the same abbreviations are then used to describe multiply-resistant staphylococci. When penicillins first became available for therapy *S. aureus* was exquisitely sensitive, which meant that its growth was inhibited at low concentrations of benzylpenicillin (approx. 0.03mg/l). This compound was used successfully in the treatment of staphylococcal septicaemia, but within a few years of its introduction into clinical use many nosocomial strains of staphylococci became resistant through the possession of β-lactamase (penicillinase). The pharmaceutical industry responded to the β-lactamase threat by devising and producing semi-synthetic β-lactams that were hydrolysed very slowly, if at all, by Gram-positive β-lactamases. The first such molecule was methicillin; other compounds with similar properties were oxacillin and its derivatives cloxacillin, dicloxacillin and flucloxacillin. However, within another four years reports appeared of clinical isolates of *S. aureus* that were resistant to methicillin and the other new penicillins—hence the expression methicillin-resistant staphylococci came into existence. By 1980 these strains had become widespread and isolates from across the world were multiply-resistant to a wide variety of other antimicrobial agents, including aminoglycosides and tetracyclines. The resistance mechanisms that have been acquired are diverse (**Figure 1**) and the genes encoding resistance have either chromosomal or plasmid locations.[1] This type of resistance profile is now found in as many as 20% of hospital isolates of *S. aureus* and in 50% or more of coagulase-negative staphylococci. Patients most at risk include those who receive prosthetic implants, organ grafts or intensive chemotherapy. Consequently, infections caused by MRSA can spread rapidly throughout intensive care wards and there have been a number of deaths reported. Hospital management procedures for such outbreaks include the closure of wards and transplant-operating theatres until the particular strain has been eradicated. Although the large amount of information encoding resistance enables MRSA to flourish in the ecological niche provided by the hospital environment, it is of little use in the general community where antibiotic pressure is virtually non-existent. In this wider environment other staphylococcal strains are present which compete successfully with MRSA strains and the latter do not survive for long periods. It may also be relevant that the genetic information encoding methicillin resistance is easily lost on storage of MRSA strains. The novel mechanism of resistance of MRSA strains to β-lactam antibiotics has provided an interesting and ongoing field of research.

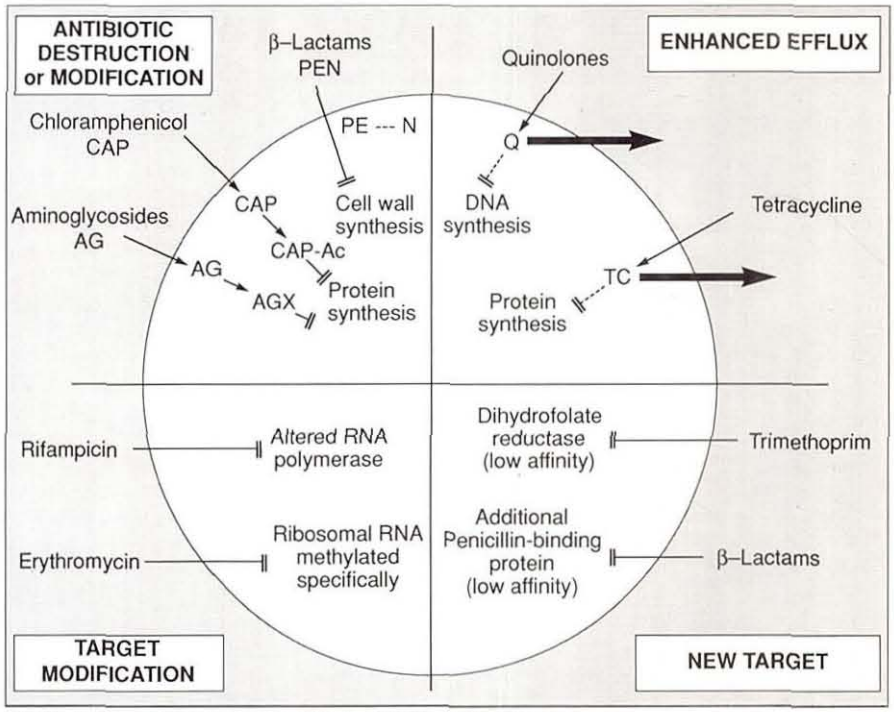


Figure 1: General mechanisms of resistance to antibiotics.

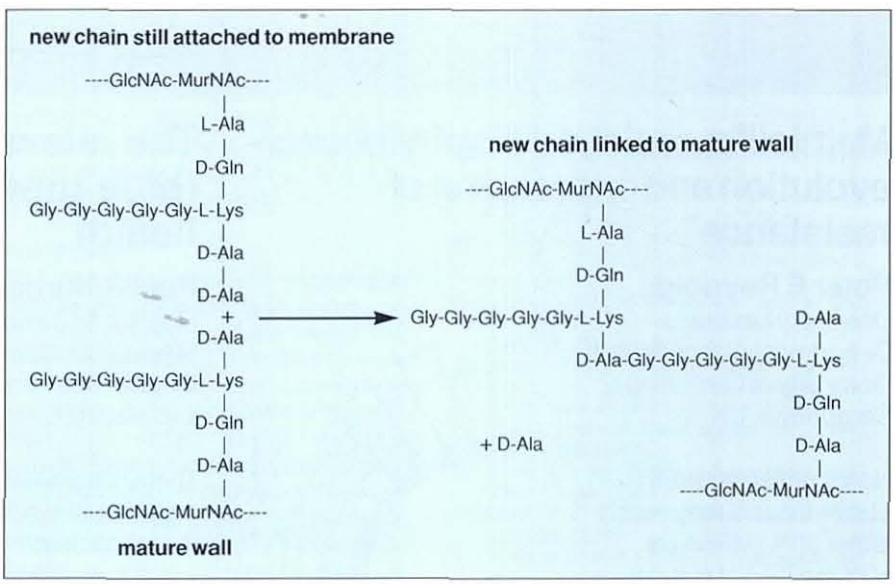


Figure 2: The peptidoglycan transpeptidation reaction in *S. aureus*.

Penicillin-binding proteins and the action of penicillin

The final stage of biosynthesis of the bacterial cell wall peptidoglycan involves a transpeptidation reaction catalysed by *penicillin-sensitive, membrane-bound proteins*. In *S. aureus* the reaction proceeds as shown in **Figure 2**.

The initial hydrolysis of the acyl-D-Ala-D-Ala bond is accompanied by acylation of the hydroxyl group of a serine residue in the active site of the enzyme: this complex then interacts with a second cell wall peptide and a cross-link is effected with release of the transpeptidase enzyme. β-lactam antibiotics interact with and bind covalently to the same serine residue in these proteins which are therefore termed penicillin-binding proteins (PBPs). The amino acid sequences of a number of PBPs that have been derived from the DNA sequences of the genes encoding them, show some conserved residues or sequences of residues in regions of the proteins that are believed to line the active site.[2] Several of these residues are critical in binding the substrate and in catalysing the transpeptidation reaction. They are also believed to be significant in interactions with β-lactam antibiotics.

General resistance mechanisms to β-lactam antibiotics

Three basically different strategies evolved by different bacterial species can result in resistance to some or all β-lactam antibiotics (**Figure 3**):

1. Several genera possess β-lactamases, enzymes that hydrolyse the β-lactam ring which in its intact state is vital for antibiotic activity. Some β-lactamases are predominantly penicillinases or cephalosporinases of narrow or wide specificity but the recent evolutionary trend in Gram-negative bacteria has involved mutations that have extended the substrate specificity to include several third generation cephalosporins. Some β-lactamases are encoded on plasmids and are inducible while others are encoded chromosomally and are produced constitutively in large amounts. Gram-positive bacteria with β-lactamases destroy β-lactam molecules in the culture medium whereas the β-lactamases of *Gram-negative organisms are secreted* into the periplasm and β-lactam molecules are destroyed after passing through the outer membrane but before interacting with the PBPs located in the cytoplasmic membrane.
2. In Gram-negative bacteria, mutations involving loss or modification of the porins (the aqueous pores through which small water-soluble molecules reach the periplasm) may decrease the rate at which β-lactam antibiotics enter the periplasm. If the periplasm also contains large amounts of a β-lactamase which hydrolyses the particular β-lactam rather slowly, the decreased rate of entry may be sufficient to change a sensitive organism into a resistant one.
3. This resistance mechanism involves a decrease in the affinity of PBPs for β-lactams as the result of changes in the geometry of the active site. The facility with which a particular β-lactam interacts with the specific serine residue of a PBP is dependent on both the PBP (the shape of the cleft and the active site) *and the shape and charge of the* β-lactam. Consequently, changes in the affinity of a vital PBP and therefore in the MIC of a β-lactam for that organism can be brought about by:
   a) multiple mutations in the active site of existing PBPs;
   b) the formation of mosaic genes in which a new gene encoding the resistant PBP is constructed from small amounts of DNA from the corresponding gene of a more resistant strain, integrated into a predominantly unchanged gene from the sensitive strain.
   c) the acquisition of a completely new, low affinity PBP, that can replace all the essential functions of the normal complement of PBPs. It is this type of mechanism that has been acquired by methicillin-resistant staphylococci.

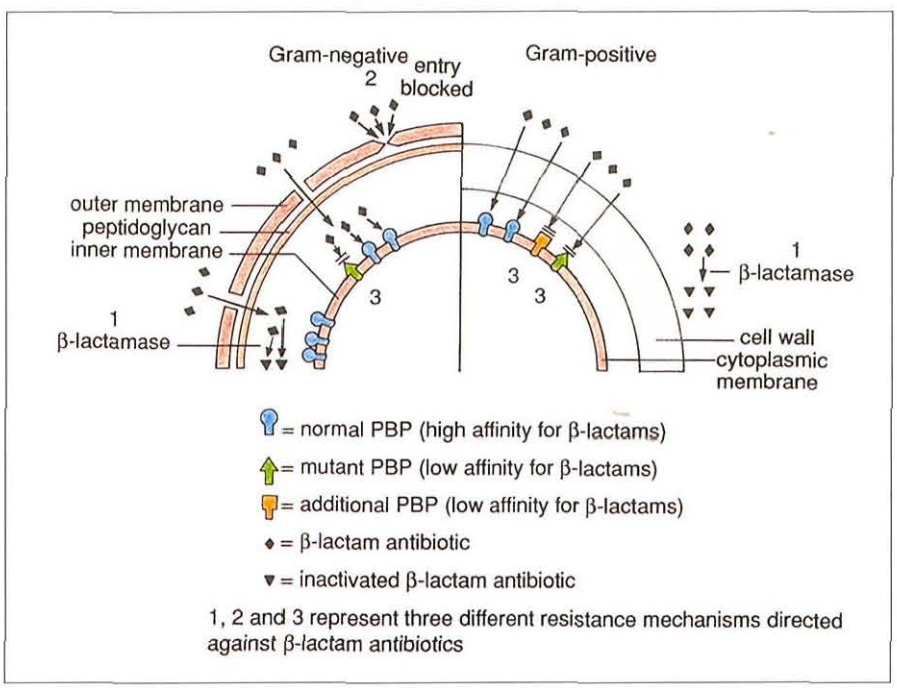


Figure 3: Mechanisms of resistance to β-lactam antibiotics.

PBP 2' or 2a

Methicillin-sensitive strains of *S. aureus contain four membrane-bound PBPs, three* of which are of high molecular weight (75,000–82,000) and which are more than 50% saturated with penicillins at concentrations close to the MIC. PBP 4 is of much lower molecular weight and is not considered essential as mutants lacking it grow normally. MRSA strains contain an additional PBP (M_T 78,000) referred to as PBP 2' or 2a because of its similar mobility to PBP 2 during the separation of membrane proteins by SDS gel electrophoresis.[3,4] It differs from the other three high molecular weight PBPs in that it has a 1000-fold lower affinity for benzylpenicillin (50% saturation occurs at 20–30mg/l, corresponding to the MIC). MRSA strains can grow in the presence of β-lactam antibiotics at concentrations that effectively saturate the normal complement of 'sensitive' PBPs and under these conditions PBP 2' is the only PBP that remains uncomplexed with β-lactams: it is, therefore, presumed to be the sole functional PBP.[4] The gene encoding PBP 2' (*mecA*) resides on additional DNA not present in isogenic sensitive strains *suggesting that the DNA encoding the new* protein has been acquired from an external source and has not been derived by mutation of a staphylococcal PBP gene. The methicillin-resistance determinant is over 30Kb long; it contains insertion sequence-like elements that enable it to integrate into a specific chromosomal site.

Analysis of PBP 2' function

Partial proteolysis of the PBP 2'-penicillin complex results in a number of peptides bound to penicillin that differ in mobility from those obtained from the corresponding complexes of PBPs 1, 2 and 3.[5] Monoclonal antibodies raised against PBP 2' do not interact with PBPs 1, 2, 3 or 4 but do interact strongly with a PBP of the same molecular weight that is present in the cell membrane of other staphylococcal species that have become methicillin resistant (e.g. methicillin-resistant strains of *S. epidermidis, S. haemolyticus, S. hominis, S. capitis*).[6] Sequencing of the DNA encoding PBP 2' from several strains of *S. aureus* and from *S. epidermidis* reveals only minor differences *between the derived protein sequences* and the portion of the protein which contains the penicillin-binding domain is identical in all the strains from which the DNA has been sequenced. These lines of evidence all suggest that PBP 2' is unique.[7]

The role of PBP 2' in wall synthesis

PBP 2' catalyses the cross-linking of newly synthesized peptidoglycan to the existing wall under non-growing conditions. This has been demonstrated by comparing the binding affinity of several β-lactam antibiotics for PBP 2' with the degree of inhibition of the transpeptidation reaction under conditions in which the other PBPs have been saturated. Furthermore, in strains in which the synthesis of PBP 2' is genetically regulated, the induction of PBP 2' is accompanied by increasing resistance of the transpeptidation reaction, and gradual

loss of PBP 2' on removal of the inducer results in increasing sensitivity of the reaction.[8] In MRSA strains expressing resistance, a normal cell wall is synthesised as indicated by analysis of cell wall fragments resulting from muramidase digestion of peptidoglycan, though the normal PBPs 1, 2, 3 and 4 are likely to remain active. When antibiotic pressure was applied (i.e. PBP 2' was probably the only active PBP remaining) the degree of cross-linkage was greatly reduced.[9] It is apparent that PBP 2' has sufficient synthetic activity to maintain the integrity of the staphylococcal wall but that it cannot completely replace the activities of PBPs 1, 2, 3 and 4 when they are inactivated.

Factors affecting expression of PBP 2'

The expression of PBP 2' is influenced both by environmental factors and by the cell genotype. Environmental factors affecting expression include the salt concentration, pH of the medium and incubation temperature. For example, PBP 2' is present in relatively small amounts after growth at 42°C in the absence of NaCl in strains that can synthesise it constitutively. As the temperature is lowered to 30°C and the NaCl content of the medium increased to 5%, the amount of PBP 2' present in the membrane increases markedly and it becomes a major membrane protein accounting for as much as 10% of the total Coomassie Blue-staining material on the SDS polyacrylamide gel.

Genetic control of PBP 2' synthesis

In the early studies with PBP 2', β-lactamase-negative isolates were used to facilitate labelling studies of the PBPs using [^{3}H]benzylpenicillin. When more recent clinical strains were examined it became clear that the synthesis of PBP 2' was under genetic control: MRSA strains containing a plasmid encoding β-lactamase, synthesised barely detectable amounts of PBP 2' unless they were grown in the presence of low concentrations of β-lactams which induced both β-lactamase and PBP 2' (**Figure 4**).[6,10] Base sequence analysis of the DNA immediately upstream of the 5' end of the *mecA* and β-lactamase (*bla*) genes demonstrated a high degree of homology and it was concluded that the *mec* gene may have been formed by a fusion between part of a PBP gene from an external source and the 5' plus upstream portions of a β-lactamase gene.[11] This could explain why synthesis of PBP 2' is inducible in strains containing the β-lactamase operon. In the uninduced state the β-lactamase repressor would interact with presumed repressor binding sites that have been identified close to the start of the structural genes and repress synthesis of both β-lactamase and PBP 2'. MRSA strains cured of the plasmid carrying the β-lactamase operon still contain the repressor binding sites but lack information for the repressor protein; therefore, synthesis of PBP 2' is constitutive.

The role of *mecR*

The inducibility of PBP 2' has also been demonstrated in MRSA strains lacking β-lactamase. A regulatory element, *mecR*, was originally detected in the methicillin resistance determinant of *S. epidermidis* that had been cloned into *Staphylococcus carnosus*, and was located approximately 1kb upstream of the *mecA* gene.[12] Hybridisation studies indicated that it was present in some but not all strains of methicillin-resistant *S. epidermidis* and *S. aureus* and that it was absent from the penicillinase plasmid p1524. Studies involving deletion mutagenesis indicated that the presence of this region of the DNA strongly repressed the synthesis of PBP 2'. Both the penicillinase plasmid and the presence of the *mecR* region of the chromosome control the level of transcription of *mecA*. It was found that *mecA* RNA was barely detectable in uninduced *mecR*-containing strains and induction was relatively slow. However, in a strain harbouring the penicillinase plasmid p1524, induction was faster and *mecA* RNA could be detected within 10 min of the addition of a β-lactam as inducer. There was also a strong correlation between the repression of PBP 2' synthesis and of methicillin resistance. It has recently been shown that the *mecR* regulatory element consists of two open reading frames which encode a transmembrane-signalling protein (MecRI) and a repressor (MecI).[13] This situation is similar to that regulating the induction of β-lactamase in *S. aureus*.

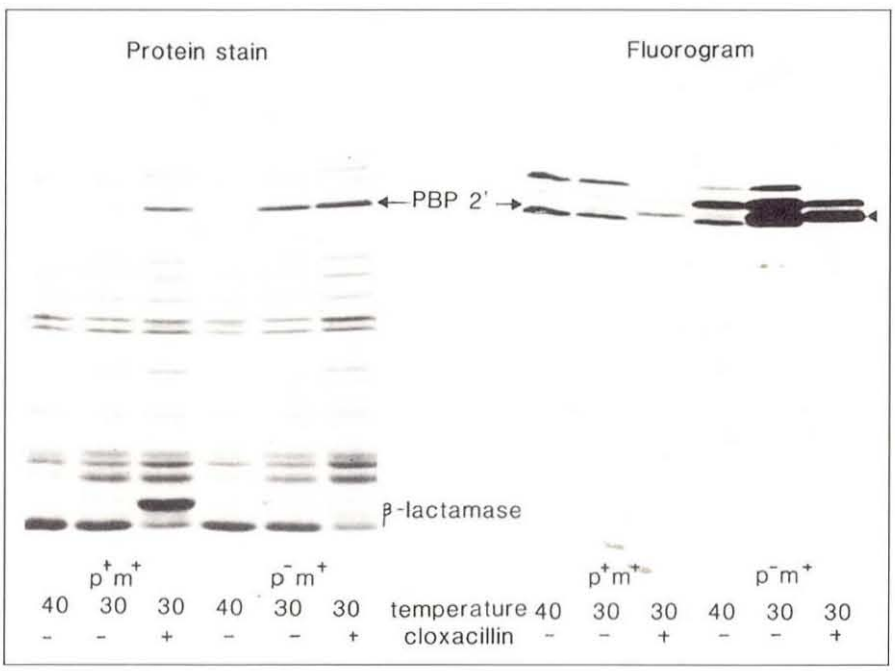


Figure 4: Effect of a plasmid containing the β-lactamase gene or growth temperature on the expression of PBP 2'. Two isogenic methicillin-resistant strains, one containing a plasmid encoding β-lactamase (p^+m^+), the other cured of the plasmid (p^-m^+), were grown under conditions described in the figure. Intact bacteria were incubated with [^{3}H]benzylpenicillin, lysed and PBPs detected by fluorography after separation and staining of membrane proteins. Strain p^+m^+ was preincubated with clavulanic acid to inhibit β-lactamase activity before labelling: clavulanic acid binds to a substantial percentage of the PBP 2' molecules which reduces the degree of labelling of this PBP.

Genetic factors affecting the expression of methicillin resistance, but not affecting synthesis of PBP 2'

FemA

Chromosomal factors unlinked to the methicillin resistance determinant are required in addition to PBP 2' for methicillin resistance to be expressed phenotypically. Transposon Tn*551* which encodes erythromycin resistance can be incorporated into the staphylococcal chromosome at many locations. If this insertion is carried out with MRSA strains, methicillin-sensitive mutants can be selected that continue to synthesize large amounts of PBP 2': one such mutant carrying the Tn*551* insertion has been termed *femA* (factor affecting the expression of methicillin resistance).[14] *FemA* encodes a 48kDa protein which is essential for the expression of high level methicillin resistance; mutants in this region have a reduced rate of cell wall turnover, a reduced rate of whole cell autolysis and the peptidoglycan synthesised is digested at a reduced rate by lysostaphin, which attacks the pentaglycine cross-bridging peptides in the peptidoglycan of *S. aureus*. The *femA* mutants have a reduced glycine content of the peptidoglycan compared with that of *femA*$^+$ strains; furthermore, introduction of a plasmid containing *femA* into a *femA*-insertionally inactivated strain, restored both methicillin resistance and the glycine content of the peptidoglycan, thus demonstrating a direct effect of *femA* on cell wall structure.[15] Detailed analysis of the peptidoglycan subunits of a *femA* mutant suggested that the defect was an inability to attach the second glycine residue to the cross-bridging peptide.[16] The effects on cell wall turnover and cell autolysis are likely to be secondary, resulting from an altered cell wall structure. The product of *femA* is not totally absent from these mutants: Tn*551* appears to be integrated into the promoter or control regions of the *femA* gene as 5–10% residual transcription was still detectable.

FemB

A second reading frame immediately downstream of *femA* may also be involved in the expression of methicillin-resistance since methicillin-resistance was not completely restored in an appropriate isolate by the addition of a construct containing only *femA*. Two other Tn*551* insertions affecting expression of methicillin-resistance which mapped near or within the 3' end of this second reading frame, could not be comple-

mented with a plasmid containing *femA*, demonstrating that a second chromosomal gene, close to *femA* is involved; this has been designated *femB*.[17,18] This gene appears to have a similar function to *femA* in that the peptidoglycan of *femB* mutants has a reduced degree of cross-linkage and fewer glycine residues are present in the interpeptide bridge compared with the parent strain.[17]

Heterogeneity of MRSA strains

It is not clear just how many genes affect the phenotypic expression of methicillin-resistance. Within a population of MRSA, resistance may be expressed homogeneously at a high level (i.e. by all cells in the population), or heterogeneously (in which two or more subpopulations of cells are present), differing in their MIC to methicillin and in the percentage make-up of the classes with a low, medium or high degree of resistance.[19] In some heterogeneous strains the frequency of cells with a high MIC may be as low as 10^{-6}–10^{-8}. This degree of non-uniformity in the expression of methicillin-resistance exists not only between strains but also within the progeny of a single MRSA isolate. The population structure in terms of methicillin-resistance is both strain specific and stable and therefore genetic control is occurring. The degree of resistance cannot be correlated with the amount of PBP 2' in the membrane: therefore the genes affecting heterogeneity do not have a direct effect on transcription of the *mecA* gene. Some of these auxiliary genes that affect expression of methicillin-resistance (e.g. *femC*) are located on the largest *Sma*1 fragment of the *S. aureus* chromosome, which also contains the *femA* and *femB* genes, while another maps in a different *Sma*1 fragment (*femD*).[18] It remains to be seen whether these other genes also affect the pathway of peptidoglycan synthesis.

Conclusions

All MRSA isolates and methicillin-resistant strains of other staphylococci contain an additional penicillin-binding protein (PBP 2') with a low affinity for β-lactam antibiotics that can apparently replace functionally the normal sensitive PBPs when they are saturated with β-lactams. The protein catalyses a transpeptidation reaction although peptidoglycan synthesised under conditions in which the other PBPs are inhibited has a low level of cross-linkage. PBP 2' is encoded on chromosomal DNA with no corresponding allele in sensitive strains and the origin of the additional DNA remains a mystery. Not all isolates containing the *mecA* gene which encodes PBP 2' express methicillin resistance. In some strains the synthesis of PBP 2' is under genetic control and at least two controlling elements have been identified. In strains lacking these control elements PBP 2' is synthesised constitutively but genes unlinked to *mecA* are necessary for the expression of resistance. The genetic factors controlling the synthesis or functioning of PBP 2' are summarised in **Figure 5**. It has been suggested that the methicillin-resistance trait is continuing to evolve. Capture of foreign DNA, leading to the formation of a PBP with a low affinity for β-lactam antibiotics, may have been followed by the recruitment of several auxiliary genes not directly affecting the amount of PBP 2' whose function results in strains with higher MICs and with greater uniformity in the expression of resistance.[19]

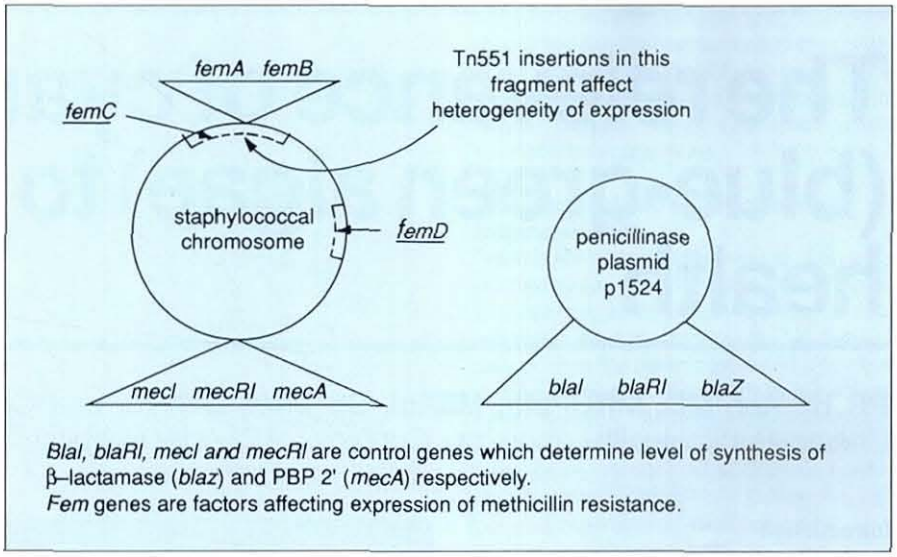


Figure 5: Genes involved in expression of PBP 2' and methicillin resistance.

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The relevance of cyanobacteria (blue-green algae) to public health

PR Hunter, MD, MRCPath, MIBiol, Dp Bact, Director and Consultant Medical Microbiologist, Chester Public Health Laboratory, Countess of Chester Hospital, Chester, UK.

Introduction

Cyanobacteria are amongst the earth's most ancient life forms. They are widespread in many aquatic environments, most notably freshwater lakes and reservoirs (**Figure 1**). Toxicity problems are usually associated with the formation of blooms or scums on the surface of the water body. Recent incidents of animal poisonings and one probable incident of human poisoning in the UK associated with cyanobacterial blooms have increased concern about their potential affects on human health.

Figure 1: Toxic algal blooms being corralled on a lake surface for subsequent disposal.

Taxonomy and biology

The cyanobacteria are true prokaryotic bacteria of the class Photobacteria. They are distinguished from many of the other Photobacteria by the presence of chlorophyll a, phycobilins, and the lack of chlorophyll b. The taxonomy of this group is complex, in part because nomenclature has followed both the Botanical Code and the microbiological codes. About 40 species of cyanobacteria have been listed in the literature as having toxic properties.[1] Those genera where toxicity is well established are *Microcystis, Anabaena, Oscillatoria, Aphanizomenon, Nodularia, Cylindrospermum, Cylindrospermopsis* and *Nostoc*.

Planktonic genera of cyanobacteria can cause toxicity problems when they form blooms or scums on the surface of a water body. About 25 genera of cyanobacteria are typically planktonic (they float in the water body).[2] To enable the cells to float, planktonic cyanobacteria contain intracellular gas vacuoles.

Cyanobacterial growth below 10°C is very poor so that blooms in temperate countries are usually restricted to the summer and early autumn. Those cyanobacteria that are usually associated with blooms, appear to prefer eutrophic lakes which are high in nutrients. This may be because other eukaryotic algae, which grow more rapidly at lower temperatures preferentially use up the available nutrients in oligotrophic waters.[2] Phosphorus seems to be the most important nutrient, some genera being able to fix atmospheric nitrogen. Indeed the apparent increase in reports of bloom formation in recent years is probably a result of human effluent causing phosphate enrichment of waters.

Bloom formation does not benefit the cyanobacterium and indeed exposes it to the potentially lethal combination of desiccation and UV light. Blooms are probably the result of maladaptation to rapidly changing meterological conditions.[2] Buoyancy is finely regulated to maintain the optimum exposure to light for photosynthetic activity. If the surface of the water body becomes more turbulent cells will be carried down into the deeper and darker zones. In an attempt to counteract this effect the cyanobacterial cells will increase their buoyancy. If the weather suddenly becomes much calmer again, the cells will not be able to reduce their buoyancy rapidly enough to stop them floating up to the surface of the water. If sufficient cells float to the surface a scum will form. The wind blowing across the surface of the water may concentrate the scum.

Cyanobacterial toxins

Most of the ill effects of cyanobacteria on animals and humans appear to be due to the production of toxins. Some 40 species of cyanobacteria have been reported to produce toxins. The diversity of the toxin producers is matched by the diversity of the toxins that have been described.[3,4]

Probably investigated in the greatest detail are the hepatotoxins, which are low-molecular-weight peptide toxins, with a seven amino acid ring. Two of these amino acids are rather unusual; N-methyldehydroalanine (Mdha), and 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (adda). This latter amino acid appears to be unique to cyanobacterial toxins. Species that have been shown to produce peptide hepatotoxins include *Microcystis aeruginosa, Nodularia spumigena, Oscillatoria agardhii, Anabaena flos-aquae* and *Aphanizomenon flos-aquae* (**Figure 2**).[4]

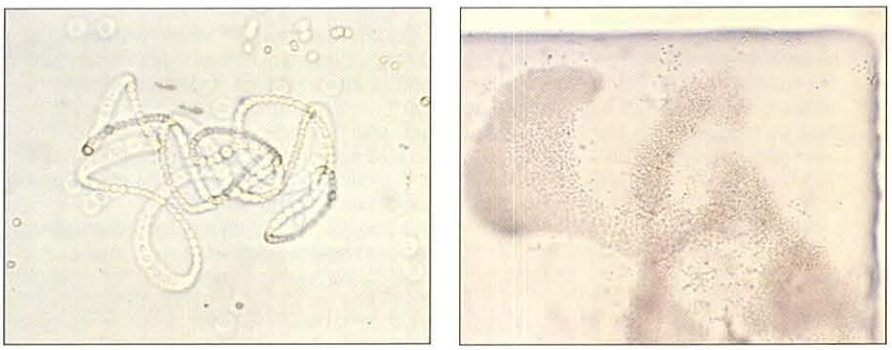

Figure 2: Microscopic morphology of *Anabaena flos-aquae* (left) and a microcolony of *Microcystis aeruginosa* (right).

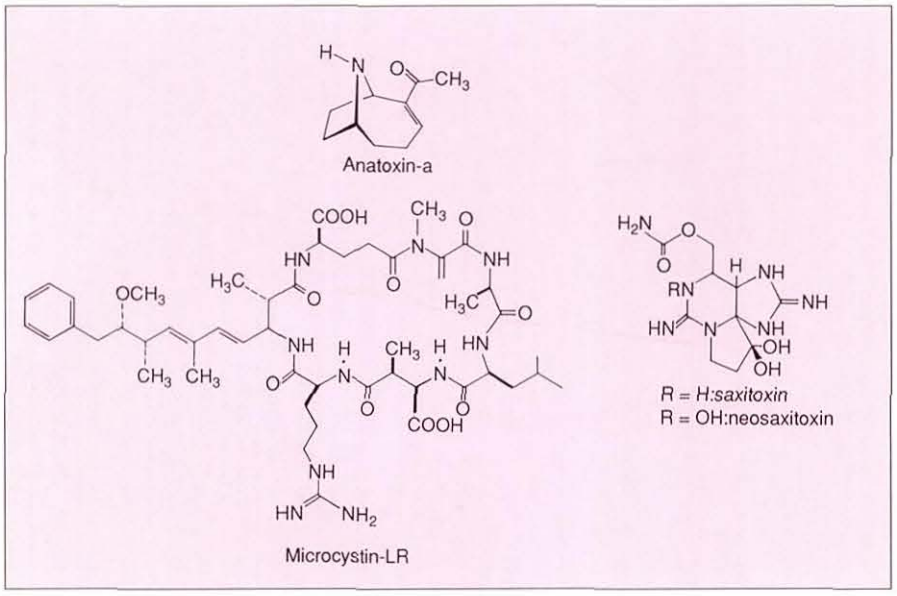

Figure 3: Chemical structure of the Anatoxins and Aphantoxins.

Six chemically related hepatotoxins have been described of which the most investigated is Microcystin LR (**Figure 3**).

Acute exposure to microcystin can cause extensive necrosis of the liver with fatal haemorrhage or functional failure.[4] Ten to 30 minutes after a lethal dose, experimental animals develop pallor, prostration, convulsions and irregular respiration. Death follows within one to three hours. The LD50 of Microcystin LR is 50µg/Kg compared to 500µg/Kg for strychnine and 10,000µg/Kg for sodium cyanide.[3] The microcystins are potent inhibitors of the protein phosphatases type 1 and type 2A.[5] There is increasing evidence that microcystins are potent tumour promoters when fed to experimental animals in sub-lethal doses. In one study mice given dilute extracts of *Microcystis* sp. developed larger and more numerous skin tumours after topical applications of dimethylbenzanthracene compared to mice given just water to drink.[6] More recent work found that microcystins are the most potent liver tumour promoters yet described.[7]

Another class of cyanobacterial toxins are the neurotoxins. The two most important are the Anatoxins and the Aphantoxins (**Figure 3**). Anatoxin-a, produced by *An. flos-aquae*, was the first such toxin to be chemically defined. It is a secondary amine (2-acetyl-9-azabicyclo-4-(4-2-1) non-2-ene) and a structural analogue of cocaine.[4,5] Anatoxin-a is a potent neuromuscular blocking agent which mimics the effects of acetylcholine.[5] The LD50 in mice is 50µg/Kg. Symptoms of anatoxin poisoning in animals include staggering, muscle fasciculations, convulsions and opisthotonos, with death due to respiratory failure.[5]

Aphantoxins were first identified in a bloom of *Aph. flos-aquae*.[5] These toxins are composed of two known alkaloid toxins, neosaxitoxin and saxitoxin, and are responsible for paralytic shellfish poisoning. The mode of action of these toxins is to inhibit nerve conduction by blocking sodium, but not potassium, transport across the axon membrane.

Cyanobacteria, like all Gram-negative bacteria, produce lipopolysaccharide (LPS) endotoxins. The cyanobacterial LPS differs from that of other bacteria in lacking phosphate in the Lipid A core and being about tenfold less toxic.[4]

Evidence of human disease linked to Cyanobacteria

Cyanobacteria and their toxins have been implicated as the cause of a variety of adverse effects in humans. Generally the route of exposure has been either by recreational water contact, by consumption of affected fish, by contamination of potable water supplies or possibly by an airborne route.[8] Possible episodes of cyanobacterial related disease will be discussed under each of these categories.

Illness associated with recreational water contact

There have been several reports of an itchy erythematous skin reaction occurring in swimmers who have been in direct contact with cyanobacterial blooms.[9–11] In some of these cases the patient exhibited a positive skin test to algal extracts compatible with an atopic reaction. Other atopic-like reactions, such as rhinitis conjunctivitis and asthma, have been reported in swimmers having contact with blooms.[12–14] Some of these patients were also skin test positive.

There have also been reports of individual cases and outbreaks of gastroenteritis after swimming in water with algal blooms.[14,15] One of the most convincing episodes was in a male physician who developed painful diarrhoea and vomiting after falling into a lake covered by an algal bloom.[15] The day after the incident he started passing green mucoid faeces. Microscopy of the faeces revealed both *Microcystis* sp. and *Anabaena circinalis*. Four days after the incident he had made a complete recovery.

More recently there was a report of two cases of atypical pneumonia in army cadets admitted to hospital after canoeing on a lake with a bloom of *Microcystis aeruginosa*.[16] These patients presented with fever, basal pneumonia, vomiting, sore throat and blistering around the mouth. Eight other recruits who had also been on the canoeing exercise complained of similar symptoms.

Possible airborne route

One study from India found that 57% of patients with asthma or allergic rhinitis gave positive skin test reactions to a variety of algal extracts.[17] No control case gave a positive skin test. Furthermore, 50% of asthmatics gave a positive bronchial provocation test to these algal extracts. Such reactivity may indicate that airborne cyanobacteria can act as an allergen in atopic lung disease.

Episodes associated with fish consumption

During the 1920s and 1930s there were many reports of a disease causing myalgia, vomiting, dyspnoea, and haematuria and some fatalities.[18] The outbreaks tended to be associated with the consumption of fish, particularly fish livers, that had been taken from lagoons with blue-green algal blooms.

Episodes associated with potable water

Several outbreaks of gastroenteritis, possibly associated with cyanobacterial toxins, have been reported in the literature.[19–21] These outbreaks have affected thousands of people. One such outbreak affected an estimated 5,000 people supplied from a single reservoir in Sewickley, Philadelphia.[21] About half of all residents taking their mains supply from the reservoir developed diarrhoea within a 24 hour period. The only potential pathogen found in the reservoir was the remains of a bloom of the potentially toxic cyanobacterium *Schizothrix calicola*. This bloom was subsequently shown to produce endotoxin.[11]

Probably the most dramatic outbreak of human illness that has been associated with cyanobacteria occurred in Palm Island, Queensland, Australia in 1979.[22] This outbreak affected 139 children and 10 adults of Aboriginal descent. The illness began as an acute hepatitis with malaise, anorexia, vomiting and tender hepatomegaly. On presentation 74% of cases had glycosuria, 89% proteinuria, 20% haematuria, and 53% had ketonuria. During the next few days 82% developed acidosis and hypokalaemia, often with severely abnormal serum electrolytes. Later 39% developed diarrhoea of which 92% passed frank blood. All cases survived, recovery taking between 4 and 26 days. The dam which provided most of the water to the community had had a bloom which was treated by copper sulphate five day before the start of the epidemic.[23] Subsequent investigations of *Cylindrospermum raciborskii*, one of the components of the bloom, were able to cause a similar disease in mice.[24] The association of human illness with either the natural or man induced death of a cyanobacterial bloom has been a feature of several outbreaks.

A retrospective study of a hospital laboratory's records of liver function tests showed that γ glutamyl-transferase levels increased in a population drinking water from a reservoir at the time of a bloom of Microcystis.[25] γ Glutamyl-transferase levels are a very sensitive marker of hepatitis. The enzyme levels returned to normal after the bloom had dispersed. A control population whose water came from another unaffected source showed no change in liver enzymes at that time. No increase in clinical disease was noted at that time.

One of the most convincing outbreaks affected patients attending a private dialysis clinic near Washington DC.[26] Twenty-three patients, on 49 occasions, developed a pyogenic reaction during a four week period corresponding with a period of a cyanobacterial bloom in the supply reservoir. These reactions were characterised by chills, fever, myalgia, nausea and vomiting, and hypotension. High levels of endotoxin were detected by the Limulus lysate test in the potable water supply. When the algal counts declined the outbreak stopped.

Figure 4: Algal blooms will be blown by the prevailing winds on to the lake shore where they may be eaten by dogs or sheep and cause illness or death.

A major concern is whether cyanobacteria or their toxins in potable water supplies are associated with cancer. Studies in animals suggest that microcystin is a potent carcinogen.[7, 27] Indeed, microcystin appears the most potent liver carcinogen yet described.[27] One epidemiological study in humans found an excess of primary liver cancer in Qidong County, north of Shanghai.[28] The people of Qidong County drink ditch water which regularly suffers from cyanobacterial blooms. The control populations drank well water. This study is by no means conclusive as ditch water could be exposed to other carcinogens such as agrochemicals.

A possible indirect carcinogenesis mechanism is *via* trihalomethanes. Several studies have linked trihalomethanes in drinking water to increased cancer incidence, particularly bladder cancer.[29–31] Cyanobacteria have been shown to be a major source of trihalomethane precursors, at least in some water supplies.[32,33]

Finally there has been the suggestion that cyanobacterial contamination of reservoirs can be associated with increased numbers of human birth defects. One epidemiological study identified an excess of human birth defects in populations supplied by two drinking water reservoirs.[34] Subsequent experimental studies found the reservoir water to be mutagenic at times of a bloom of *Oscillatoria subbrevis*.

Conclusions

There is a great wealth of experimental evidence that cyanobacterial toxins can cause severe adverse health effects. This experimental evidence is backed by many observations of animal deaths after exposure to cyanobacterial blooms (**Figure 4**). Despite the strong animal evidence, epidemiological evidence of human ill health is often anecdotal and incomplete. The exact risk that cyanobacteria pose to public health will only be determined if appropriate prospective epidemiological studies are undertaken.

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