The new threats of Gram positive pathogens: re-emergence of things past

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...re-emergence of things past,
I sigh the lack of many a thing I sought,
And with old woes new wail my dear times' waste.

Sonnet No.30 (W Shakespeare, adapted)

Introduction

In recent years Gram positive bacteria have re-emerged as important pathogens, both in the community and hospital. To compound the problems, antimicrobial resistance, long considered the domain of Gram negative bacteria, is being increasingly exhibited by Gram positive strains.

This short review will look at renewed interest in previously uncommon pathogens "our old friends"—*Corynebacterium diphtheriae* and *Streptococcus pyogenes*; and the serious problems we face in treating patients infected with *Staphylococcus aureus*, *Streptococcus pneumoniae* and the enterococci.

Streptococcus pyogenes

Before the discovery of antibiotics, serious infections caused by *S pyogenes* were common and were responsible for as many as 50% of post-partum deaths and the major cause of deaths in patients with burns before World War II.¹ The infectious sequelae, such as rheumatic fever, were also common. The introduction of penicillin resulted in the organism being consigned to the history books. But not for long.

Since the mid-1980s an increased incidence of invasive disease has been reported from many parts of the industrialised world, including the USA, the UK and Scandinavia.²⁻⁴ Coincidentally, focal resurgence of rheumatic fever was reported in different areas in the USA, beginning in the Rocky Mountain area.⁵⁻⁶ The predominant serotypes associated with rheumatic fever were M 1, 3 and 18, which produce characteristic mucoid colonies. Changes in the prevalence of predominant serotypes continue to occur, which may cause the observed fluctuation in the incidence of streptococcal disease. In the UK, types 1, 28 and 4 now predominate,⁷ whilst in the USA types 1, 3 and 8 are prevalent.² These new M types are more invasive, cause increased mortality and are more likely to occur in clusters. This increased invasiveness may be related to the low level of immunity in populations where these M types are less common. There is also evidence that patients with serious disease are infected by a unique clone.⁸ Of those studied, 90% had a characteristic restriction fragment length profile and were positive for the streptococcal pyogenic exotoxin (SPE) A gene.

Until recently, streptococcal isolates from cases of scarlet fever were only positive for SPE-B or SPE-C.⁹ Strains which produce SPE-A are now being isolated from patients with widespread cellulitis or necrotising fasciitis, associated with severe systemic toxicity, resembling staphylococcal toxic shock syndrome.¹⁰ Strains isolated before 1940 were also positive for SPE-A; so it seems that the more toxic form of scarlet fever was prevalent prior to the introduction of antibiotics, and is now re-establishing itself.

The term streptococcal toxic shock-like syndrome (TSLS) has been used to describe patients with hypotension and multisystem failure typical of the staphylococcal disease.¹¹ This phenomenon can be partly explained by the fact that SPE-A shows 50% amino acid homology with staphylococcal enterotoxin B, one of the main toxins responsible for staphylococcal toxic shock syndrome.¹² The clinical outcome of TSLS depends on the interaction between the microbial virulence factors (M protein and SPEs) and the immune status of the host.⁴

Penicillin remains the treatment of choice for streptococcal infection, but in patients with severe disease large numbers of the organisms can be found, and the efficacy of penicillin is thereby reduced. Two explanations have been proposed; the slower growth rate of streptococci when present in large numbers¹³ or the decreased expression of penicillin binding proteins (PBPs) in the presence of large inocula.¹⁴ Other antibiotics which have been found to be more effective in experimental animal models include clindamycin and erythromycin.¹⁵ The possibility of a future multivalent group A streptococcal vaccine has recently received a boost, following reports that a purified tetravalent M protein produced significant antibody levels against all four serotypes of native M proteins in a rabbit model.¹⁶

Staphylococcus aureus

S aureus has been well known as a major pathogen for many years. It causes septicaemia, endocarditis, osteomyelitis, abscesses, pneumonia, wound infections, impetigo, boils, and a variety of toxin mediated diseases. In the pre-antibiotic era *S aureus* bacteraemia carried a mortality of approximately 82% and despite the availability of effective antibiotic treatment, mortality remains high at 25-63%. In recent years *S aureus* has been newly implicated in further diseases such as toxic shock syndrome¹⁷ and Kawasaki syndrome.¹⁸ However, it is in the field of hospital infection control that *S aureus* is causing increasing concern due to the emer-

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gence of increasingly resistant strains with high epidemic potential and virulence.

S aureus was identified as a cause of hospital acquired wound infection in the 1880s. As streptococcal wound infections decreased in incidence *S aureus* became the commonest cause of nosocomial wound infection in the 1940s. In 1980 the first national survey of infections in hospital identified *S aureus* as responsible for 18% of all nosocomial infections and 33% of postoperative wound infections in the UK.¹⁹

S aureus has displayed a great ability to develop resistance to a wide range of antibiotics. Soon after the introduction of penicillin the first resistant strain producing β -lactamase was reported²⁰ and penicillin resistance rapidly spread. In the early 1960s β -lactamase stable penicillins such as flucloxacillin were developed which have become the mainstay of antibiotic treatment for *S aureus* in the UK. In the laboratory flucloxacillin sensitivity of *S aureus* is inferred from the sensitivity to the related compound methicillin. Resistance to methicillin is mediated by alterations to the PBP_s on the cell surface which confers cross-resistance to all β -lactams including cephalosporins.

Strains of *S aureus* resistant to methicillin (MRSA) were reported as early as 1961²¹ and were involved in nosocomial infection by 1966.²² During the 1970s little notice was taken of MRSA—most strains were not multiply resistant to other classes of antibiotics and outbreaks of infection seemed to be manageable. During this time there was some suggestion that MRSA was inherently less virulent than methicillin sensitive *S aureus* (MSSA). However, not surprisingly, it transpires that strains of MRSA vary in their virulence and epidemic potential just as MSSA.

Interest in *S aureus* was re-kindled in the early 1980s with the recognition that a single strain was causing nosocomial outbreaks of infection in a number of hospitals in the south-east of England. A survey in 1988 identified 14 strains of MRSA which were affecting more than one hospital in England and Wales.²³ These strains were termed epidemic MRSA (EMRSA) strains; EMRSA-1 (the SE England strain) was by far the most common, affecting 50 hospitals.

With the recognition of uncontrolled spread of EMRSA, infection control guidelines were published and have been updated.²⁴ However, these have not been successful in controlling the problem. This is partly due to the nature of modern health provision with increased inter-hospital referral, increased reliance on agency nursing staff who may transfer between many hospitals, increased numbers of severely debilitated inpatients, and partly due to new emergent strains of MRSA with high epidemic potential. Since 1990 two new epidemic strains have emerged, EMRSA-15 and EMRSA-16. By June 1995, these strains were each affecting more than 80 hospitals.²⁵ Of particular concern is EMRSA-16 which is resistant to many anti-staphylococcal antibiotics including erythromycin, gentamicin, neomycin, ciprofloxacin, and trimethoprim. EMRSA-16 is generally

sensitive to mupirocin but some isolates exhibit resistance. Mupirocin resistance is a problem as nasal mupirocin is the most effective and recommended treatment of MRSA carriers.²⁴

At present, the mainstay of treatment for infections caused by MRSA is vancomycin. This antibiotic has been used for more than three decades without the development of resistance in *S aureus*. However, there is concern that vancomycin resistance may emerge. The related glycopeptide teicoplanin is often used instead of vancomycin as it has a better side effect profile. Worryingly, there have been reports of the failure of teicoplanin treatment for *S aureus* infection due to the emergence of teicoplanin resistance.²⁶ Resistance to vancomycin has been reported in coagulase negative staphylococci²⁷ and in enterococci (discussed later). Vancomycin resistance is still difficult to select for in vitro but transfer of resistance genes from vancomycin resistant enterococci (VRE) to *S aureus* has already been achieved in the laboratory.²⁸ With the increasing use of vancomycin for treating MRSA infections and the spread of VRE in the same hospital population it would seem likely that there will be transfer of resistance between the genera. When this occurs in the patient population we shall return to the pre-antibiotic era for *S aureus*.

Enterococci

Enterococci are found as normal gastrointestinal and vaginal flora, and are some of the most frequently isolated organisms from clinical specimens. In general, they are considered to be of low pathogenicity, and are much less frequently found as a cause of infection. In adults enterococci are particularly associated with infection of the urinary tract. They are also a well recognised cause of endocarditis, and bacteraemia may result from instrumentation of the urinary or gastrointestinal tract, or from infection of indwelling vascular lines.

There is evidence that enterococcal infections are becoming more common, particularly in immunocompromised patients, and they are now a major cause of nosocomial infection.²⁹ This is predominantly infection related to invasive procedures, although selection pressure in favour of enterococci through antibiotic use, particularly cephalosporins, is important. The relative antibiotic resistance of enterococci makes treatment difficult, but the ability of enterococci to acquire new resistance is also a major cause for concern.

Enterococci are intrinsically less sensitive to β -lactam antibiotics than streptococci because they possess low affinity PBP_s, and are generally regarded as being resistant to cephalosporins.³⁰ However, a combination of penicillin or ampicillin and aminoglycoside is synergistic and has become the treatment of choice for serious enterococcal infection. *E faecium* seems to be more resistant than *E faecalis*; higher minimum inhibitory concentrations (MIC) to penicillin are seen (typically 8–32 v 1–2 mg/l), and recently *E faecium* showing high level resistance to gentamicin (MIC >2000

mg/l) have become common, accounting for >50% of isolates in some centres. These strains do not show synergy between β -lactam antibiotics and aminoglycosides. High level resistance to β -lactam antibiotics has also emerged (penicillin MIC >200 mg/l). This may be because of the production of β -lactamase (particularly in *E faecium*, where β -lactamase production and high level resistance to aminoglycosides are commonly seen together), or (in *E faecium* and *E raffinosus*) result from overproduction of a low affinity PBP (PBP-5).^{30 31} Acquired resistance to chloramphenicol, macrolides, cotrimoxazole, and tetracycline may also occur.³⁰

It is therefore understandable that there was considerable concern when initial reports of VRE appeared in 1988.^{32 33} Since then, isolation of VRE has become more common and geographically widespread. VRE accounted for 14% of enterococci in intensive care units in the USA in 1993.²⁹ Many VRE, particularly isolates of *E faecium*, also show high level resistance to β -lactam antibiotics and aminoglycosides.

Based on phenotypic characteristics, three main groups of resistance have been described: VanA, VanB and VanC.³⁴ The VanA phenotype is inducible high level resistance to both vancomycin and teicoplanin. Resistance is readily transferable and is conferred by a plasmid gene designated *vanA*, encoding an altered peptide in peptidoglycan synthesis which prevents binding of glycopeptides to the cell wall.³⁴ VanB resistance is also inducible and transferable, but the exact site of the *vanB* gene is unknown. It confers moderate resistance to vancomycin, but not teicoplanin. Like VanA resistance, it is most commonly seen in *E faecium*, but also occurs in *E faecalis*. VanA resistance may also occur in *E avium*. VanC resistance is constitutively expressed and the *vanC* gene is likely to be chromosomal. It is seen in *E gallinarum* and *E casseliflavus* and confers low level resistance to vancomycin alone.

The epidemiology of VRE is not completely understood, but the apparent rapid spread of these organisms is of great concern. The recent emergence of VRE not only jeopardises treatment of enterococcal infection, but threatens the potential spread of resistance to other organisms, particularly staphylococci. Risk factors for infection with VRE include inpatients of large hospitals (>500 beds), length of hospital stay (>2 weeks), severe underlying disease, haematological malignancy, and recent treatment with antibiotics, including vancomycin.^{29 35 36} Nosocomial transmission of VRE within hospitals is well documented.^{37 38}

Infection control measures to limit the spread of VRE have been published recently.^{39 40} These include increased surveillance for vancomycin resistance, measures to limit the unnecessary use of glycopeptides, and control measures to prevent spread within the hospital environment. It has been suggested that "because of the grave clinical problems posed by VRE, herculean measures (to prevent spread) seem eminently appropriate".³⁹

Pneumococci

Streptococcus pneumoniae has been known as a major pathogen for over 100 years. Despite effective antimicrobial chemotherapy it continues to cause considerable morbidity and mortality: *S pneumoniae* causes approximately 25% of community acquired pneumonia, of which if severe enough to require admission to an intensive therapy unit, the causative organism is most likely to be the pneumococcus.⁴¹ *S pneumoniae* accounted for 10% of isolates from blood cultures and 18% of isolates from cerebrospinal fluid reported to the PHLS Communicable Disease Surveillance Centre (CDSC) in 1982–92⁴²; *S pneumoniae* causes up to 50% of episodes of otitis media and is a major cause of bacterial sinusitis and bronchitis.

Recently, there has been increasing concern over pneumococcal disease because of the increasing spread of penicillin resistant pneumococci. A pneumococcus is defined as intermediately resistant to penicillin if the strain has a MIC >0.1 mg/l but <1.0 mg/l and fully resistant if the MIC is >2.0 mg/l. Penicillin resistance was first selected in laboratory strains of pneumococcus soon after the release of penicillin in 1941. However, it was not until 1967 that a clinically significant pneumococcus with reduced penicillin sensitivity (MIC 0.6 mg/l) was reported in an immunodeficient patient with pneumonia in Australia. In 1978 multiply resistant pneumococci with high level penicillin resistance were reported in South Africa.⁴³ In the late 1970s penicillin resistant pneumococci were most prevalent in New Guinea, Israel, Poland, Spain, and South Africa, but they are now found in increasing numbers worldwide.⁴⁴ For example, 41% of isolates from children in Atlanta, USA, in 1994 were penicillin resistant⁴⁵; penicillin resistance in France rose from less than 1.1% in 1984 to 12% by 1990⁴⁶; the incidence of penicillin resistant pneumococci in children in a university hospital in Korea in 1991–3 was 82%.⁴⁷ By comparison, only 2.5% of reported isolates from bacteraemia or meningitis reported to the PHLS CDSC in 1993 were resistant to penicillin.⁴⁸

Resistance to penicillin in pneumococci is achieved by alteration of the high molecular weight PBPs.⁴⁹ Genetic studies suggest that penicillin resistance in pneumococci has arisen through natural transformation and genetic recombination with other penicillin resistant species such as *Streptococcus mitis*, *Streptococcus sanguis* or *Streptococcus mitior*. There is a great deal of diversity among strains of pneumococci resistant to penicillin, but superimposed on this diversity there seem to be some clones with increased epidemic potential. For example, it seems that a clone of serotype 23F displaying multiple drug resistance has spread from Spain and South Africa to the USA.⁵⁰

The impact of penicillin resistance on the severity of disease has yet to be fully established. The main risk factors for infection by penicillin resistant pneumococci are similar to the risk factors for severe pneumococcal disease—extremes of age, hospitalisation, and

previous antibiotic treatment.⁵¹ As initial empiric treatment is likely to be ineffective it is not surprising that the outcome in some patients is poor. However, a study from Barcelona has suggested that the mortality due to pneumococcal pneumonia is not affected by the penicillin susceptibility of the pneumococcus.⁵²

Treatment of infections caused by penicillin resistant pneumococci has been reviewed elsewhere.⁵³ With intravenous treatment the concentrations of penicillin in the serum that can be achieved will greatly exceed the MIC of intermediately resistant and many fully resistant strains. Thus, a β -lactam remains the empiric drug of choice in this situation. However, if a highly resistant strain is suspected, vancomycin or a carbapenem should be considered. In the case of meningitis, penicillin is unlikely to be effective and the empiric therapy would be a third generation cephalosporin such as cefotaxime. Unfortunately, there have been reports of failure of cefotaxime treatment in meningitis due to penicillin resistant pneumococci that are also resistant to third generation cephalosporins.⁵⁴ In this situation vancomycin or rifampicin, or both, has been suggested. Recent results show that such resistance to third generation cephalosporins is rare in the UK, with a prevalence of <0.2%.⁵⁵

As noted earlier, some clones of pneumococci seem to have increased epidemic potential and it is likely that the prevalence of penicillin resistant pneumococci in the UK will increase. If treatment is less effective there should be increased impetus for the development of a conjugate vaccine and a re-assessment of the indications for vaccination and of our empiric antimicrobial treatment.

Diphtheria

Classic pharyngeal diphtheria results from infection with exotoxin producing strains of *Corynebacterium diphtheriae*. The importance of diphtheria lies, firstly, in the mortality of 5–10% of those infected (which has not changed greatly over the past 50 years), and, secondly, in the potential for person to person spread of the organism. In the UK diphtheria is now uncommon. Notification in children under the age of four years in London fell from approximately 100 cases per 10 000 children in 1935 to one case per 10 000 in 1950.⁵⁶ This followed introduction of diphtheria toxoid and increasing uptake of vaccination from 5% in 1935 to 77% in 1950. Improved socioeconomic conditions have been important also. Vaccine uptake in the UK is currently 94%.⁵⁷ Between 1986 and 1993 only 33 toxigenic *C. diphtheriae* isolates were reported in England and Wales, the majority of which were imported from the Indian Subcontinent.⁵⁸

There have been several recent changes, however, which are a cause for concern, and emphasise the importance of this traditional infectious disease. Since 1991 the incidence of pharyngeal diphtheria in countries of the former Soviet Union has increased dramatically. WHO figures show that there has been an increase in reported cases from 3100 in 1991 to 46 000 in 1994 (table 1).⁵⁹ Several factors

Table 1 Reported cases of diphtheria in countries of the former Soviet Union (figures from the WHO)

Year	No. of cases reported
1991	3100
1992	5700
1993	19 500
1994	46 000

have contributed to this epidemic, related to the social and economic changes occurring in the former Soviet Union at the time. For a variety of reasons, diphtheria immunisation rates fell in the 1980s, so that by 1990 only 68% of Russian children had been vaccinated against diphtheria.⁵⁹ Also, immunity in previously vaccinated adults may have fallen to sub-protective levels, and natural immunity was poor because of the low incidence of diphtheria at the time. Therefore, the population was susceptible to infection when diphtheria was introduced by movement of large numbers of people following political changes in the early 1990s. The epidemic continued because of shortages of diphtheria vaccine and difficulties in improving vaccine uptake.

This increasing incidence of diphtheria is of concern to other countries. While most imported cases originate in either the Indian Subcontinent or Africa, diphtheria from the former Soviet Union has occurred in some European countries, but not, as yet, in the UK. It is known that some developed countries have low vaccine coverage. Also, protective antibody levels are not present in a significant proportion of previously vaccinated adults, particularly women, and therefore there is potential for further epidemic spread to occur if diphtheria is reintroduced.^{60–61} The WHO Expanded Program on Immunisation in the European Region has stressed the importance of vaccination, and has set a target of 95% coverage in every country by 1995.⁶² In addition it has been suggested that a booster dose of diphtheria vaccine should be given to school-leavers.⁶³ The importance of effective diphtheria surveillance and immediate control measures has also been emphasised.

There is also concern about disease caused by non-toxigenic *C. diphtheriae*. The current status of *C. diphtheriae* carriage in the UK is unknown. As clinical diphtheria is now so uncommon many microbiology laboratories no longer routinely look for the organism in throat swabs, and it is very possible that its isolation from other sites would be overlooked. Despite this, isolation of non-toxigenic strains of *C. diphtheriae* has been more frequently reported.⁶⁴ Increased pharyngeal carriage has been documented in homosexual men attending a genito-urinary clinic in London⁶⁵ and in intravenous drug users.⁶⁶ Invasive infection due to non-toxigenic *C. diphtheriae* is well described, and typing has shown that clusters of infection have often been caused by single strains.^{67–68} This would be a particular cause for concern if these strains were able to become toxin producers. Lysogenic conversion to toxin production is possible in vitro,⁶⁹ but its clinical significance is unknown.

Conclusions

The resurgence of these Gram positive organisms is unquestionable and resistance to multiple antimicrobial agents continues apace. We need to be aware of these threats and hope that suitable alternatives to the classic or standard therapeutic agents are forthcoming.

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