



Infections caused by Gram-positive bacteria: a review of the global challenge

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Summary Infections caused by multidrug-resistant Gram-positive bacteria represent a major public health burden, not just in terms of morbidity and mortality, but also in terms of increased expenditure on patient management and implementation of infection control measures. *Staphylococcus aureus* and *Enterococcus* spp. are established pathogens in the hospital environment, and their frequent multidrug resistance complicates therapy. The archetypal hospital “superbug”, methicillin-resistant *S. aureus* (MRSA), regularly attracts mass-media interest and, in many countries, there is political pressure to reduce MRSA infection rates, with some progress now being made in the United Kingdom and the United States. To compound these established problems, we have witnessed the emergence and spread of virulent clones of MRSA in the community, and of *Clostridium difficile* in hospitals. Multidrug-resistant *Streptococcus pneumoniae* clones are major community pathogens in many parts of the world, but are now being challenged by new conjugate vaccines. Using combinations of molecular epidemiological tools, which characterize the resistant isolates and their resistance determinants, scientists can track highly successful bacterial strains at local, national, and international levels. These methods have provided new insights into the evolution of key pathogens, and this information may aid the design of control strategies and vaccines. In addition, the development of new antimicrobials – including oxazolidinones, lipopeptides, glycolcyclines, ketolides, and new generations of fluoroquinolones, antistaphylococcal β -lactams, and glycopeptides – must remain a high priority for the continued effective treatment of infections caused by resistant strains. So far, resistance to these newer agents is identified rarely in surveillance programs, but occasional reports of resistance causing therapeutic failure (e.g., with linezolid, daptomycin, telithromycin, or newer fluoroquinolones) give cause for concern. The emergence of antibiotic resistance is inevitable, but we must seek to decrease its impact and prolong the effectiveness of the agents available to us.

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1. Introduction

Penicillin was introduced into clinical use in the 1940s and revolutionized the treatment of infections caused by Gram-positive bacteria, especially staphylococci and streptococci. In the subsequent 60 years, many further antibacterial agents have been launched in an unending struggle to keep ahead of our bacterial adversaries, which have consistently shown their adaptability by developing resistance to almost every agent with which they have been challenged. Among Gram-positive pathogens, *Staphylococcus aureus*, *Streptococcus pneumoniae* and, more recently, enterococci, each present global resistance challenges, causing significant public health concern and adding to the cost of health care. This article provides an

overview of our current knowledge of the epidemiology of these bacteria, with emphasis on findings gained through application of molecular techniques. It also reviews the nature of their antibiotic resistance, which frequently extends to multiple drug classes, occasionally including newly-introduced agents. Indeed, multidrug resistance is usually the primary and most pressing indicator of problematic strains; in this respect, little has changed since reports of staphylococci resistant to penicillin, tetracycline, and streptomycin in the 1950s.¹

2. Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin was introduced into clinical use in 1960 and was closely followed by the first reports of MRSA,² with their resistance arising through the production of a supplementary penicillin-binding protein (PBP), known as PBP2' or PBP2a. This compensates for the functions of the essential methicillin-susceptible staphylococcal PBPs and confers resistance to most β -lactams, although novel cephalosporins

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and carbapenems, such as ceftobiprole, ceftaroline, and PTZ-601 bind to it and so retain antistaphylococcal activity.^{3,4} PBP2' is encoded by *mecA*, which is located within a much larger DNA element, the staphylococcal chromosome cassette *mec* (SCC*mec*). SCC*mec* elements are categorized into at least eight types and various subtypes.^{5–7} They vary in size from ~20 kb (type IV and VI elements) to >60 kb (type III elements), but *mecA* (~2 kb) itself represents only a small proportion of the SCC*mec* element, while the remainder includes *mec* regulatory sequences, recombinase genes (*ccr*), insertion sequences (e.g. IS431), and transposons (e.g. Tn554). They may also include other resistance determinants, and even linearized plasmids.^{8–11} SCC*mec* elements integrate into a specific position (*attB* or *intM*) of the *S. aureus* chromosome,^{8,12} within a gene known as *orfX*. This insertion site is very consistent, and PCR assays that target the junction between the acquired SCC*mec* element and the *S. aureus* chromosome have been exploited for the rapid detection of MRSA directly from clinical samples, such as nasal swabs.^{13–15} A close homologue of *mecA* has been identified in *Staphylococcus sciuri*,¹⁶ and can confer methicillin resistance when transferred into *S. aureus*.¹⁷ This may point to the route whereby MRSA (and methicillin-resistant strains of other *Staphylococcus* spp.) originally acquired *mecA*. However, the ability of SCC*mec* elements to transfer horizontally between strains is not readily demonstrable *in vitro*; rather, the evidence for its movement is epidemiological, and includes the variety of SCC*mec* elements acquired by *S. aureus*,^{8–11} the presence of identical SCC*mec* elements in different clonal lineages of *S. aureus*^{7,18} and coagulase-negative staphylococci, and the loss *in vivo* of *mecA* or SCC*mec* from particular "MRSA" strains.^{19,20} Historically, *S. aureus* strains were defined by phage typing, antibiograms, and other biological characteristics.^{21–23} Molecular methods such as pulsed-field gel electrophoresis (PFGE) have been applied more recently to outbreak investigation because of their greater discrimination.^{24–26} Using combinations of traditional and molecular methods, it was possible to determine the local and national epidemiology of MRSA, and national "epidemic strains" were defined in many countries; it was also possible to identify several strains that had spread between countries. Nevertheless, the comparative typing methods available until the 1990s did not readily lend themselves to tracing international spread of successful lineages unless the isolates were centrally collected and compared directly. This changed with the development of a scheme for multi-locus sequence typing of *S. aureus* (MLST; <http://www.mlst.net>).²⁷ The underlying concepts and advantages of MLST have been reviewed elsewhere;²⁸ the method is universally applicable to any isolate in any laboratory with sequencing capability, and a definitive type can be assigned (rather than a comparative profile, as provided by PFGE, for example), using a Web-hosted database to interpret the allelic profile obtained by sequencing standardized, short fragments of (typically) seven loci.

MLST has been used to investigate the evolution of MRSA strains. Enright et al. went further, by combining sequence type (STs) data with data for the types of SCC*mec* elements so as to define clones of MRSA; a clone being

defined as a group of isolates that have the same ST and the same SCC*mec* type.¹⁸ Using this approach, it was shown conclusively that multiple different ST lineages have acquired the same SCC*mec* elements, confirming that these elements do transfer. Distinct MRSA clones have emerged from some genetic backgrounds on more than one occasion so that MRSA isolates can have the same ST but differ in their SCC*mec* type. Many of the major national epidemic MRSA strains affecting hospitals belong to evolutionary lineages that have spread internationally.^{7,18} The prevalence of MRSA rose markedly in many countries in the past two decades, leading to major control efforts. In the United Kingdom, the proportion of MRSA among all cases of *S. aureus* bacteremia rose sharply in the 1990s, peaked at 40–45% (equivalent to 7,000–9,000 cases per annum) during 2001–2005, but fell sharply to 36% in 2007 following implementation of strategies prompted by targets set by the UK government (http://www.hpa.org.uk/infections/topics_az/hai/Mandatory_Results.htm; <http://www.rivm.nl/earss>). Much of the initial increase was due to the rise to dominance of two epidemic MRSA strains, EMRSA-15 (ST22-MRSA-IV) and EMRSA-16 (ST36-MRSA-II),^{18,29,30} with EMRSA-15 still accounting for the majority of all MRSA from bacteremias in the United Kingdom. Elsewhere in Western and Southern Europe, MRSA rates now widely exceed 25%, with rates of approximately 50% reported in 2007 in Greece, Cyprus, Malta, and Portugal. MRSA remains much less prevalent (<2%) in The Netherlands and Scandinavia (Fig. 1; <http://www.rivm.nl/earss>), reflecting the success of rigorous "search and destroy" infection control. Nevertheless, the MRSA rate has increased even in The Netherlands: the Berlin clone (ST45-MRSA-IV¹⁸) emerged in 2000 and, by 2002, had been replaced by a new clone (ST45-MRSA-"new") with a novel SCC*mec* element.³¹ Many isolates of these clones were not highly resistant to oxacillin (MICs 4–32 mg/L), meaning that they were not detected reliably in clinical laboratories.³¹ Such detection difficulties may allow colonized patients to "slip under the net" of "search and destroy" infection control policies.³² More recently, The Netherlands has witnessed human infections, including

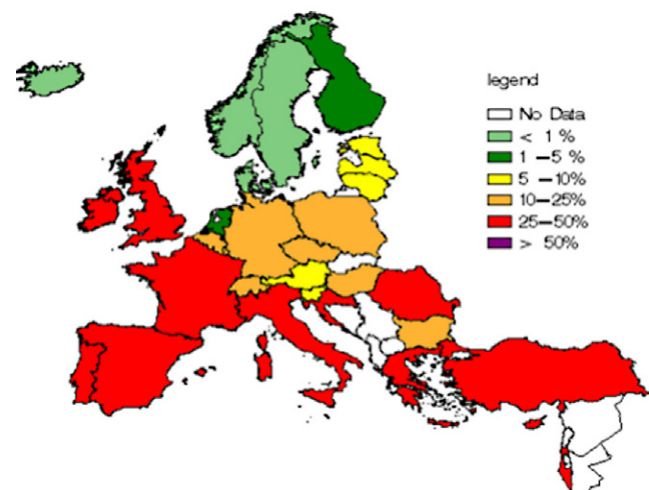


Fig. 1 – Proportions of MRSA isolates from bacteremias reported in 2007 by countries participating in the EARSS surveillance scheme.

hospital outbreaks, caused by the livestock (pig)-associated ST398 MRSA clone,^{33–35} which has also been reported elsewhere. In the United States, the prevalence of MRSA is even higher than in much of Europe; 2004 data from the LEADER Program, which collected Gram-positive clinical isolates from 50 US laboratories in 33 states, found a prevalence of 54.2%.³⁶ Data from the LEADER Program from 2007 recorded a similar rate, 58.1%.³⁷

2.1. The emergence of community-associated MRSA (CA-MRSA)

MRSA poses new threats and challenges beyond the hospital with the emergence of CA-MRSA,^{38,39} which are associated with infections in patients without recent history (and in some instances, without any history) of hospital admission and without the classical risk factors for MRSA carriage (including health care contact or nursing home residency). Rather, outbreaks of CA-MRSA infections have affected nursery children, teams of contact sportsmen, prisoners, military recruits, and men having sex with men.⁴⁰ They have become most prevalent in the United States and remain rare in Europe and Asia. CA-MRSA strains have only recently acquired *SCCmec* types IV and V. In addition – and of major significance – many of these strains have increased virulence through production of the Pantone-Valentine leukocidin (PVL) toxin, which is encoded by bacteriophages. CA-MRSA strains are most often associated with skin and soft-tissue infections, usually in young healthy individuals; more rarely, these strains also cause life-threatening necrotizing pneumonia.^{40,41} Molecular analysis has revealed that the prevalent CA-MRSA strains are distinct from clones established in the health care setting, as defined by PFGE, MLST, and other molecular typing tools.^{7,42} Nevertheless, some strains may represent the re-emergence of old *S. aureus* strains that have more recently acquired *SCCmec* variants. A good example is the emergence of PVL-producing CA-MRSA strain ST30-MRSA-IV, also known as the Southwest Pacific (SWP) clone⁴³ or USA1100,⁴⁴ in the south-west Pacific region (including Australia), the United States, and in Europe. This strain is related to the highly virulent phage type 80/81 methicillin-susceptible *S. aureus* clone (ST30-MSSA), which was globally pandemic in the 1950s and which also produced PVL toxin. Both ST30-MSSA and ST30-MRSA-IV have branched from an *S. aureus* lineage that also yielded the PVL-negative epidemic hospital strain ST36-MRSA-II, prevalent in the United Kingdom and also known as EMRSA-16. This therefore suggests initial acquisition of PVL genes, followed by acquisition of the *SCCmec* type IV determinant⁴³ (Fig. 2). The evolutionary origins of the major MRSA clones and the possible relation between CA-MRSA and health care associated MRSA have recently been reviewed.⁷ Genome analysis of the prevalent CA-MRSA strain USA300 (ST08-MRSA-IV) shows that it is highly related to the very first known MRSA, strain COL.^{45,46} Many CA-MRSA strains are less broadly resistant to antimicrobials than are health care associated strains. Most epidemic hospital MRSA strains are resistant to β -lactams (owing to the *mecA*-encoded PBP2'), to fluoroquinolones, and frequently also to other antibiotic classes, including macrolide-

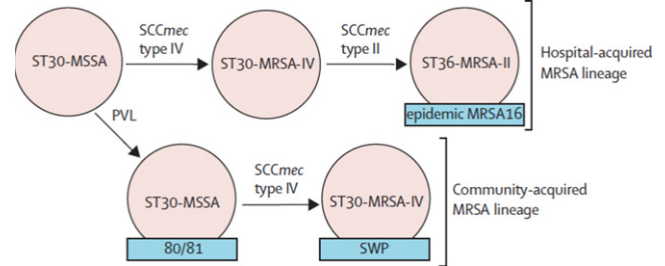


Fig. 2 – Model for the evolution of ST30 and its descendants. MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; *SCCmec*, staphylococcal chromosome cassette *mec*; PVL, Pantone-Valentine leukocidin. Reproduced with permission from Robinson DA, et al. *Lancet* 2005;365:1256–8.⁴³

lincosamide-streptogram B (MLS_B) agents, tetracyclines, and aminoglycosides. In contrast, many CA-MRSA strains are resistant only to β -lactams, and probably represent recent acquisitions of *SCCmec*.⁴⁶ Ciprofloxacin susceptibility has been used as a marker of a CA-MRSA strain, although it is neither absolutely sensitive nor specific, especially outside of the United States. Some CA-MRSA strains, such as ST80-MRSA-IV in Europe, including the United Kingdom, are additionally resistant to tetracyclines and to fusidic acid, while the US strain USA300 is resistant to macrolides, owing to an *msr(A)* determinant on a 30-kb penicillinase plasmid.⁴⁴ As always, caution must be exercised when using antibiograms to define strains as they are prone to change; some isolates of the USA300 strain have acquired 4.3-kb and 2.6-kb plasmids, which carry *tet(K)* and *erm(C)* genes, respectively.⁴⁴ Epithets that imply a clear distinction between health care associated versus CA-MRSA may be artificial or only temporarily useful. There is concern that PVL-producing CA-MRSA strains are moving into the health care setting.^{47,48} Seybold et al.⁴⁹ reported that the USA300 strain was responsible for 28% of health care associated MRSA bacteremias in an urban public hospital in Atlanta. It seems likely that these “new” PVL-positive MRSA strains are as fit as established health care associated strains, and able to compete with them in some or all nosocomial infection types. If so, we might expect strain replacement in health care settings in the next few years. This would be troubling owing to the high prevalence of PVL in the new strains, enhancing their virulence. For this reason, we should perhaps regard MRSA strains as PVL-positive or -negative, rather than as health care associated or community-associated, though it may be too late to reverse current usage, as “CA-MRSA” is established in the scientific literature and in the minds of clinicians and microbiologists, albeit with different definitions among authors.⁴⁰ For the clinical diagnostic laboratory, there is no simple, definitive way of distinguishing CA-MRSA and hospital MRSA, although, as noted, ciprofloxacin susceptibility may be a useful indicator. For reference laboratories, analysis by a combination of several methods is preferred. PFGE is the standard method used to define MRSA strains from suspected outbreaks in the United States and elsewhere,^{25,50} but should be supplemented with MLST/*SCCmec* typing¹⁸ or *spa* typing.⁵¹

2.2. MRSA with reduced susceptibility or resistance to vancomycin

In addition to new virulence, MRSA continue to develop new resistances. Treatment for serious MRSA infections has conventionally relied on glycopeptides. However, MRSA with reduced susceptibility to vancomycin (MICs 8 mg/L) were reported in Japan in 1997⁵² and subsequently from several other countries.^{53–55} These isolates are labelled with the acronym VISA or GISA (vancomycin-intermediate *S. aureus*/glycopeptide-intermediate *S. aureus*) based on the intermediate level of resistance relative to breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). The VISA/GISA acronym serves to distinguish isolates with this low-level resistance from true vancomycin-resistant *S. aureus* (VRSA), which have acquired an enterococcal *vanA* determinant.

VISA/GISA strains have been well-characterized phenotypically, though less so at the genetic level. They show increased cell wall thickness⁵⁶ and an increased proportion of glutamine nonamidated mucopeptides,^{57,58} leading to decreased cross-linking of peptidoglycan and increased proportions of D-alanyl–D-alanine termini.⁵⁹ These termini are the binding target of glycopeptides, and increases in their proportion lead to sequestration (“trapping”) of glycopeptide molecules. The genetic basis of these phenotypic changes has not been fully defined, though it is clear that the resistance entails chromosomal mutations and does not result from the acquisition of *van* determinants from enterococci. The precise genes involved have not been identified,⁶⁰ although disruption of the *agr* accessory gene regulator is believed to play a significant role.⁶¹ In addition, the importance of mutations in the two-component regulatory systems *vraSR* and *graSR* has recently been demonstrated in the prototype VISA strain Mu50.⁶²

The first true VRSA (vancomycin MIC, >128 mg/L; teicoplanin MIC, 32 mg/L) was confirmed in 2002,⁶³ and was isolated in Michigan (MI-VRSA) from a diabetic foot ulcer, which also yielded vancomycin-resistant *Enterococcus faecalis*. At least six more VRSA have since been reported from the United States,⁶⁴ four in Michigan, one in Pennsylvania (PA-VRSA),⁶⁵ and one in New York.⁶⁶ Levels of glycopeptide resistance vary among these isolates; the MICs of vancomycin and teicoplanin for PA-VRSA were only 32 mg/L and 8–16 mg/L, respectively, although it also harbored an enterococcal *vanA* determinant.⁶⁷ This difference in level of resistance between VRSA isolates is attributed to instability of the *vanA* determinant in PA-VRSA leading to high frequency loss, together with a much longer lag phase before induction of the resistance genes.⁶⁸ More recently, VRSA have also been reported from India⁶⁹ and Iran.⁷⁰

Glycopeptide resistance is more frequently observed in coagulase-negative staphylococci, particularly in *S. epidermidis* and *S. haemolyticus*, but is usually restricted to teicoplanin. Data from the BSAC Bacteraemia Surveillance Programme for the United Kingdom and Ireland indicate prevalence rates of teicoplanin nonsusceptibility in coagulase-negative staphylococci from bacteremias of 9.1% and 26.5% among methicillin-susceptible and -resistant isolates, respectively.⁷¹ Most of the resistant

isolates required teicoplanin MICs of 8 mg/L, considered intermediate; the BSAC breakpoint for susceptibility is 4 mg/L, with resistance defined as an MIC > 8 mg/L. The MIC for one isolate of *S. haemolyticus* was 64 mg/L. All of the teicoplanin-nonsusceptible isolates remained susceptible to vancomycin in vitro (MICs ≤ 4 mg/L). Of 496 coagulase-negative staphylococci collected from various clinical specimens in the United States as part of the LEADER Program (2004), vancomycin resistance was noted in a single isolate of *S. haemolyticus* from blood³⁶; teicoplanin was not tested as it is not licensed in the United States. A similarly low incidence of vancomycin resistance was noted in 2007 in the LEADER Program.³⁷

3. Multi-resistant enterococci

Unlike *S. aureus*, enterococci are not primary pathogens. Rather, they are normal components of the human bowel flora and are not problematic for healthy people, except as occasional agents of urinary tract infections. However, the genus has proven adept at causing opportunist infections in hospital patients. These are mostly caused by *E. faecalis*, though infections caused by *E. faecium* give greatest clinical concern because of greater multidrug resistance in this species.⁷² All enterococci are resistant to available cephalosporins (except some *E. faecalis* to ceftobiprole), and are nonsusceptible to aminoglycosides. They are also relatively resistant (compared with streptococci) to penicillins, with tolerance developing rapidly upon exposure.⁷³ Until recently, no single agent could be anticipated to exert strong bactericidal activity against an *Enterococcus*, and the established therapy for serious infections remains a synergistic combination of a cell wall-active agent plus an aminoglycoside (usually gentamicin). A major problem is the ready ability of enterococci to acquire resistance horizontally, which frequently compromises the efficacy of this regimen; resistance to either component negates the bactericidal effect.

Transferable resistance to penicillins is rare in enterococci. Rather, most high-level ampicillin/penicillin resistance results from the overproduction of a low-affinity PBP, PBP5. This trait is almost always observed in *E. faecium*, where the majority of isolates now show resistance (MIC > 8 mg/L) or high-level resistance (MIC > 100 mg/L). Moreover, the *pbp5* gene is located on the chromosome and can be mobilized by transposons (e.g., *vanB* transposon Tn5382/1549) if these insert downstream of it and then excise inaccurately, taking the *pbp5* gene with them.⁷⁴ Transmission of *pbp5* has been reported at low frequencies (~10⁻⁹) from several diverse *E. faecium* strains, some of which lacked *vanB* transposons.⁷⁵ A β -lactamase related to staphylococcal penicillinase has been described in a few enterococci, mostly *E. faecalis*,^{76,77} but also in occasional isolates of *E. faecium*.⁷⁸ Producer strains were first reported in the late 1980s in the United States,^{79,80} where they caused several nosocomial outbreaks,^{81–83} and in Argentina.⁸⁴ The *bla* gene was located on plasmids or, in some strains, on the chromosome;^{85,86} in most cases, it was genetically linked with high-level gentamicin resistance. Despite this brief flourish, and for reasons that

are unclear, β -lactamase-producing enterococci have not become established as hospital pathogens.

Enterococci may acquire determinants conferring resistance to many antibiotic classes,⁸⁷ but those responsible for high-level resistance to aminoglycosides and glycopeptides cause greatest clinical concern. Many aminoglycoside-modifying enzymes have been described in the genus, but the bifunctional acetyltransferase/phosphotransferase, AAC(6')-APH(2''), which confers resistance to all members of the class except streptomycin, and, in some instances, arbekacin, which is only available in Japan and Korea, is the most troublesome. The gene that encodes this enzyme is associated with transposable elements, which may be located on plasmid or chromosomal DNA; it is also widely responsible for gentamicin resistance in staphylococci. In most countries in Europe, the prevalence of high-level aminoglycoside resistance in both *E. faecalis* and *E. faecium* from bacteremias exceeds 25% and in several countries, exceeds 50% (<http://www.rivm.nl/earss>). During 2006, the prevalence of high-level gentamicin resistance among enterococci from bacteremias in the United Kingdom and Ireland was 30.9% in *E. faecium* and 47.9% in *E. faecalis*, with the rate in *E. faecium* having fallen from >45% recorded in each of the three preceding years.⁸⁸

The prevalence of glycopeptide-resistant strains is lower than for high-level gentamicin resistance in Europe, and with a much stronger bias towards *E. faecium*. During 2006, the prevalence of vancomycin resistance in the United Kingdom and Ireland was 32.1% for *E. faecium* and 2.8% for *E. faecalis*.⁸⁸ Similar rates have been reported for Cyprus, Germany, Greece, Italy, Portugal, and Israel (<http://www.rivm.nl/earss>). In most other European countries, the prevalence of glycopeptide-resistant *E. faecium* remains <10%. EARSS data for *E. faecalis* in 2007 indicate the highest rate of glycopeptide resistance in Greece (6.7%), with other countries reporting <5% resistance (<http://www.rivm.nl/earss>). The epidemiology of glycopeptide resistance in enterococci across Europe has recently been reviewed.⁸⁹

The United States has much higher rates of glycopeptide-resistant enterococci than anywhere in Europe; the LEADER Program (2004) identified vancomycin resistance in 72.4% of *E. faecium* and in 9.6% of *E. faecalis*³⁶ collected from a variety of clinical specimens. In the LEADER Program (2007), vancomycin resistance was recorded for 30% of 705 enterococci (no species breakdown was available).³⁷

The molecular epidemiology of resistant enterococci has been investigated extensively and is complicated by horizontal transmission of resistance determinants, sometimes during the course of otherwise clonal outbreaks.⁹⁰ There are at least six acquired types (VanA, VanB, VanD, VanE, VanG and VanL), and a seventh type (VanC) that is intrinsic to *E. gallinarum* and *E. casseliflavus/flavescens*.⁹¹ The major glycopeptide resistance phenotypes of concern are VanA (with high-level resistance to vancomycin and cross-resistance to teicoplanin) and VanB (with variable levels of vancomycin resistance but retained susceptibility in vitro to teicoplanin). Strains with VanA resistance predominate globally, although VanB strains may be locally prevalent. Many of the host strains of *E. faecium* are not only resistant to glycopeptides, but also to ampicillin and to high levels

of aminoglycosides, severely restricting treatment options. Linezolid generally remains active, while quinupristin-dalfopristin is active against *E. faecium* but not *E. faecalis*. Daptomycin has borderline activity at present dosage regimens, with *E. faecalis* typically more susceptible than *E. faecium*. As yet, EUCAST has not set breakpoints for the genus because "There is insufficient evidence that the species in question is a good target for therapy with the drug" (<http://www.srga.org/eucastwt/mictab/MICdaptomycin.html>). Daptomycin does, however, show bactericidal activity against enterococci in vitro,^{92,93} and may prove useful in settings (such as endocarditis) where this is important. High-dose trials to investigate the utility in this setting are under way.⁹⁴ The unlicensed, second-generation glycopeptides oritavancin^{92,95} and telavancin⁹⁶ are also often bactericidal against enterococci. Resistance to these newer agents remains rare among clinical isolates but has been documented (see below). In general, though, they remain borderline at best against VanA strains with "activity" contingent on what breakpoints are set. Quinupristin-dalfopristin-resistant *E. faecium* can be isolated from animals fed the related streptogramin virginiamycin, and also from uncooked meat.

PFGE has also been the gold standard for molecular typing of enterococci for outbreak investigation but, as with staphylococci, is more useful for comparative than definitive typing. Standardized criteria for the interpretation of PFGE banding patterns and for defining strains have been produced,⁹⁷ but these must be used with caution as sometimes they may be too stringent for investigation of outbreaks of enterococcal infections if these extend over long periods or multiple centers. In some instances, the degree of variation is such that PFGE fails to detect relationships among isolates that appear highly related by other typing methods.⁹⁸

In the mid-1990s, vancomycin-resistant *E. faecium* isolates from across the United Kingdom were compared to investigate the possible existence of epidemic strains, which were defined as those isolated from at least two patients in each of two (or more) centers. Five such epidemic strains were identified, restricted primarily to large teaching centers in the London area, with few instances of inter-hospital spread beyond London attributable to patient transfers.⁹⁹ Since then, the national epidemiology of glycopeptide-resistant enterococci in the United Kingdom has not been investigated to any comparable extent, although local studies have been published.^{100,101} There is evidence, however, that strains of enterococci continue to spread between UK centers: In particular, a significant association was noted between high-level gentamicin resistance (plasmid-mediated) and ciprofloxacin resistance (mediated by chromosomal mutation) in *E. faecalis* isolates from bacteremias, and two clusters of related isolates were identified by PFGE, with each found in multiple centers.¹⁰² Interestingly, similar clonal spread of gentamicin/ciprofloxacin-resistant *E. faecalis* has also been reported in intensive care units in Sweden,¹⁰³ though these were not compared with those circulating in the United Kingdom.

These data, and a huge number of reports of hospital outbreaks in many countries, prove the importance of

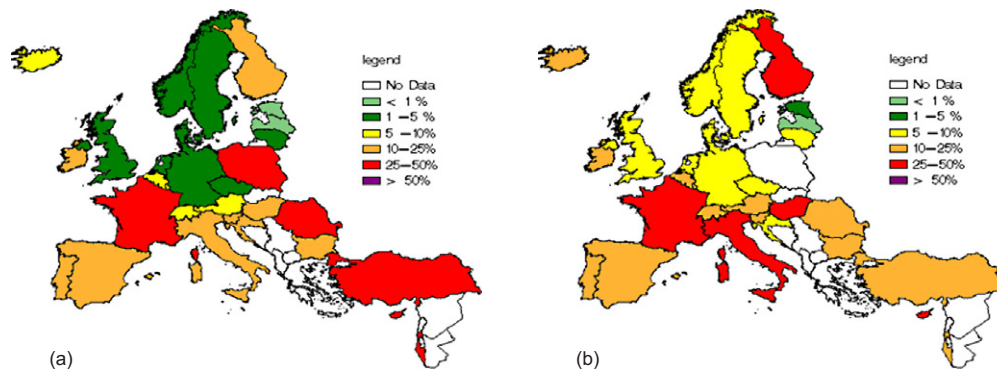


Fig. 3. Proportions of (a) penicillin nonsusceptible and (b) erythromycin nonsusceptible isolates of *S. pneumoniae* from bacteremias reported in 2007 by countries participating in the EARSS surveillance scheme.

clonal spread of resistant enterococci. This is consistent with the view expressed over 15 years ago by Jett et al.¹⁰⁴ that some strains “have heightened capabilities to colonize, overgrow, invade host tissues and persist, compared with endogenous enterococcal flora.” The extent of this clonality has been made even clearer in recent years, following the development of definitive MLST schemes for *E. faecium*¹⁰⁵ and, more recently, for *E. faecalis*¹⁰⁶ (both accessible through <http://www.mlst.net>). Comparison of many glycopeptide-resistant *E. faecium* strains from around the world has shown that most strains causing hospital outbreaks are distinct from sporadic clinical isolates, and from glycopeptide-resistant strains commonly isolated from animals. Perhaps more surprisingly, many of the outbreak strains, though distinct from each other by PFGE, represent expansion of a particularly successful clone. This group of related sequence types, which is known collectively as clonal complex-17 (CC17), is hospital adapted, ampicillin resistant and has a putative pathogenicity island¹⁰⁷ and high-level ciprofloxacin resistance.¹⁰⁸ Willems et al.¹⁰⁷ concluded that “Complex-17 is an example of cumulative evolutionary processes that improved the relative fitness of bacteria in hospital environments.”

4. Penicillin- and macrolide-resistant *Streptococcus pneumoniae*

In the United Kingdom, the prevalence of penicillin-nonsusceptible respiratory isolates of *S. pneumoniae* in 1999–2007 was 7.8% for intermediate resistance (MIC, 0.1–1 mg/L) and 0.3% for full resistance (MIC \geq 2 mg/L).¹⁰⁹ Moreover, and interestingly, the prevalence of nonsusceptible isolates from bacteremia decreased from 4.1% in 1999 and 2000 to <2% in 2003–2007 (<http://www.rivm.nl/earss>). The highest rates of penicillin nonsusceptibility among bacteremia isolates in EARSS during 2007 were reported from Cyprus (6.7% resistance, 26.7% intermediate), France (4.2% resistance, 29.9% intermediate), Israel (7.3% resistance, 22.0% intermediate), Romania (23.1% resistance, 7.7% intermediate), Poland (9.5% resistance, 19.0% intermediate), and Turkey (10.8% resistance, 17.1% intermediate) (Fig. 3a; <http://www.rivm.nl/earss>). In the United States, the LEADER (2004) Program reported prevalence rates of 14.7% penicillin resistance and 18.5% intermediate resistance among clinical isolates from diverse clinical

sources,³⁶ with comparable rates of nonsusceptibility recorded in the LEADER 2007 Program,³⁷ and also from the PROTEKT surveillance of isolates from community-acquired respiratory tract infections (Fig. 4). As in the United Kingdom, there is evidence of a decline in the prevalence of fully resistant isolates in the United States; rates fell from 26.3% to 16.5% between 2000 and 2004, although the prevalence of intermediate resistance increased from 12.5% to 20.0%.^{110,111}

In the United States between 2000 and 2004, the PROTEKT data showed an overall prevalence of erythromycin-resistant pneumococci of 29.3%, but with geographic variation (Fig. 4).^{110,111} Most resistant isolates (>60%) carried a *mef(A)* macrolide efflux determinant; however, the proportion of isolates carrying this resistance mechanism alone decreased, while the proportion also carrying an *erm(B)* gene increased from 9.7% to 18.4% of erythromycin-resistant isolates.^{110,111} In Europe during 2007, the highest prevalence rates of macrolide resistance among isolates from bacteremias were reported from France (35.6%), Hungary (35.0%), Italy (28.2%), Belgium (24.5%), and Finland (24.5%) (Fig. 3b; <http://www.rivm.nl/earss>). Data from the BSAC Surveillance Programme indicate the prevalence of macrolide resistance among pneumococci in the United Kingdom and Ireland is 12% (respiratory isolates) to 14% (isolates from bacteremia).¹⁰⁹

As with MRSA and multidrug-resistant enterococci, the spread of resistant *S. pneumoniae* clones has been monitored using traditional and molecular approaches. The Pneumococcal Molecular Epidemiology Network (PMEN; <http://www.sph.emory.edu/PMEN>) was established in 1997 to track the international spread of multidrug-resistant pneumococcal clones. Clones are named according to a standard format, e.g., Spain^{23F}-1, indicating Spain as the country in which the clone was first identified (based on publication); ^{23F} as the serotype of the clone when first identified; and 1 as the clone number 1 (assigned by PMEN). If members of a single clone are found to express a capsular polysaccharide that differs from that originally reported (e.g., as a result of transformation of capsular genes), this is also indicated, e.g., as Spain^{23F}-1-19F (indicates a serotype 19F variant of the Spain^{23F}-1 clone). At least 43 resistant pneumococcal clones have been formally recognized¹¹² (<http://www.sph.emory.edu/PMEN>), with the validity of each confirmed by MLST, PFGE, and by PCR-

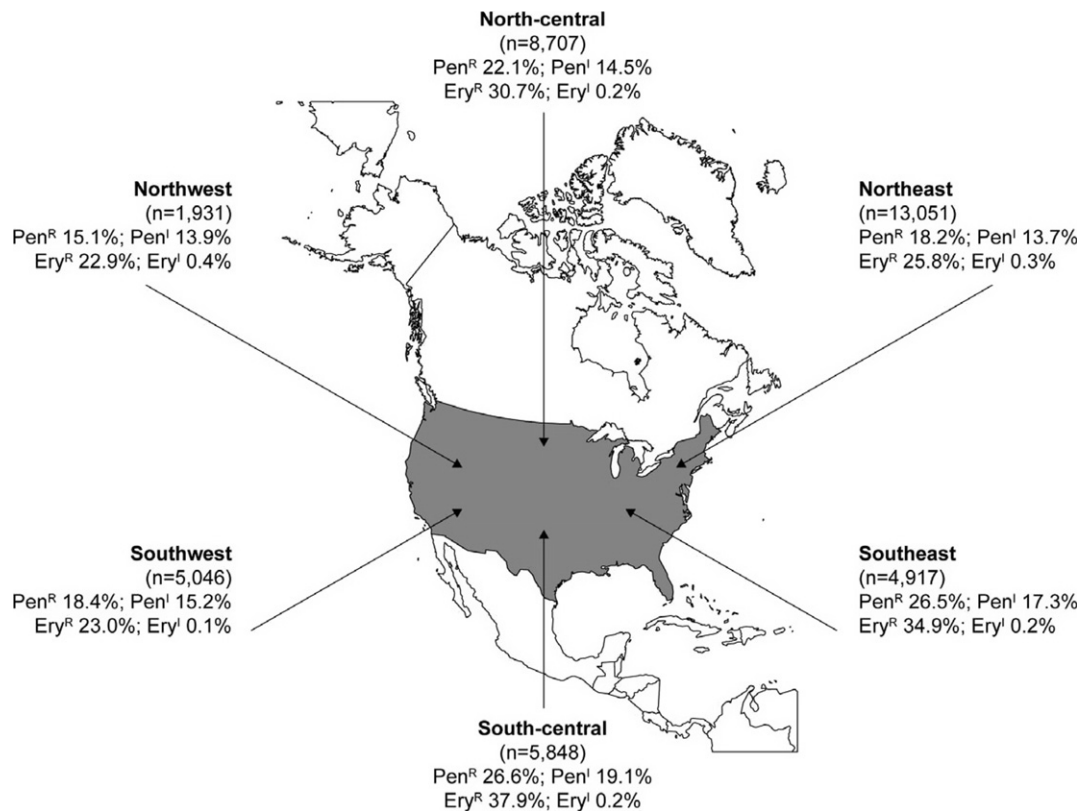


Fig. 4. Geographic distribution of rates of penicillin and erythromycin nonsusceptibility in isolates of *S. pneumoniae* collected during the PROTEKT US study Years 1–4 (2000–2004). Pen^R, penicillin-resistant; Pen^I, penicillin-intermediate; Ery^R, erythromycin-resistant; Ery^I, erythromycin-intermediate. Reproduced with permission from Jenkins SG, et al. *Ann Clin Microbiol Antimicrob* 2008;7:1.¹¹¹

RFLP analysis of the *pbp1a*, *pbp2b*, and *pbp2x* genes. The international spread of each clone, with relevant citations, is detailed on the PMEN website.

It is tempting to speculate about the underlying causes of the declining prevalence of fully penicillin-resistant isolates noted in the United Kingdom and the United States. While this is likely to be multifactorial, there is a positive correlation between national rates of penicillin resistance and prescribing of β -lactams,¹¹³ which suggests that reduced community antibiotic usage¹¹⁴ may be one component. However, the prevalence of erythromycin resistance in the United Kingdom is relatively stable despite declining usage. In the United States, the decline may also reflect deployment of the seven-valent conjugate vaccine, which covers serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.¹¹⁵ This is immunogenic in children 2 months of age and older, and provides coverage against many of the serotypes most associated with antibiotic resistance, whereas earlier 23-valent vaccines were only suitable for adults (http://www.hpa.org.uk/infections/topics_az/pneumococcal/guidelines.htm). Elsewhere, this new vaccine has been deployed rather later. Transformation, which was crucial for the evolution of penicillin resistance in pneumococci,^{116–118} enables isolates of old pneumococcal clones to switch their serotype following transformation of relevant capsular genes. Thus, serotype switching provides one route whereby pneumococci could evade vaccine coverage.^{119–121} There is also potential for replacement of resistant strains with serotypes covered

by the vaccines by new resistant strains with other serotypes. A penicillin clone with intermediate resistance (MICs 0.25–2 mg/L) belonging to serotype 35B emerged as a cause of invasive disease in the United States from 1995 to 2001, with isolates from at least 10 geographically scattered states.¹²² Subsequently, this clone has become disseminated internationally, with reports concerning carriers in Swedish daycare centers¹²³ and children in Israel with acute otitis media.¹¹⁵ In the study of children in Israel, three other prevalent clones were penicillin-nonsusceptible (mostly with intermediate resistance) and of nonvaccine serotypes, 33F, 21, and 15B/C.¹¹⁵ Even greater diversity of novel, penicillin-nonsusceptible serotypes has been reported in HIV-infected adults and their children in Kenya.¹²⁰ The spread of a highly penicillin-resistant serotype 19A, which is not covered by the vaccine, is perhaps the biggest concern.¹²⁴

5. Resistance to new agents: the Red Queen marches on!

In the 1980s, the pharmaceutical industry responded to the resurgence of MRSA and the emergence of glycopeptide-resistant enterococci by developing new agents with anti-gram-positive activity. The results of these efforts are a considerable number of new agents belonging to multiple drug classes both licensed and in development. However, resistance is as close as we can get to a “Darwinian inevitability” (if such a thing exists): a response by

bacteria to adverse environmental conditions. Resistance has emerged to all currently used antibiotics, and this unfortunate reality will continue. The Red Queen^{87,92,125} keeps marching, and we must strive to stay ahead.

Linezolid has excellent activity against the vast majority of MRSA and other *S. aureus* strains, with MICs clustered in a narrow, unimodal range from 0.5 to 4 mg/L.^{36,126} However, resistance may emerge – albeit rarely – during therapy, arising via mutations in chromosomal genes encoding 23S ribosomal RNA. Two mutations have been reported in the genes of clinical MRSA isolates, G2576T^{127,128} and T2500A,¹²⁹ although mutations at other positions have been observed in laboratory-generated mutants.^{130–132} As this form of resistance is not transferable between strains, the major public health concern is that linezolid resistance will emerge in a successful lineage and that the spread of this variant between patients or centers will not be adequately controlled. It is ominous in this regard that linezolid resistance mediated by the G2576T mutation has emerged in isolates belonging to both of the two major epidemic MRSA lineages in the United Kingdom: EMSRA-15 (ST22-MRSA-IV)¹³³ and EMRSA-16 (ST36-MRSA-II).¹³⁴ Cross-infection of four patients on an intensive care unit with a linezolid-resistant strain of *S. epidermidis* was reported recently.¹³⁵

Linezolid resistance has emerged during therapy more often in enterococci^{136–140} though mostly in protracted therapy, which is discouraged owing to the risk of hematological adverse events. There are reports of hospital transmission of resistant strains^{141,142} and we are aware that linezolid-resistant *E. faecium* isolates have been isolated from multiple patients in two UK hospitals; these isolates represented a single strain as defined by PFGE, suggesting inter-hospital spread (unpublished data). As in *S. aureus*, oxazolidinone resistance in enterococci is most commonly mediated by a G2576T mutation in 23S rRNA genes, although, again, further mutations have been observed in vitro.¹⁴³ Enterococci and staphylococci have multiple copies of these genes and emerging linezolid resistance entails a two-step process with an initial mutation introducing T2576 into one 23S rRNA gene copy, followed by intrachromosomal recombination (gene conversion), which then distributes the mutation to sufficient other gene copies to confer phenotypic resistance.¹⁴⁴ The number of mutated rDNA gene copies correlates with the level of resistance displayed.^{145–149} It is interesting to note that gene conversion can also redistribute wild-type rRNA gene copies, at least in vitro, where serial passage of a linezolid-resistant clinical *S. aureus* isolate in the absence of linezolid resulted in its reversion to susceptibility, with the MIC reduced from 12 to 2 mg/L. Nevertheless, one rRNA gene copy retained the T2576 mutation,¹⁴⁹ potentially permitting the rapid re-emergence of resistance under selective conditions. Interestingly, mutations to ribosomal protein L4 were recently reported to confer reduced linezolid susceptibility (MIC 4 mg/L) in two clinical isolates of *S. pneumoniae*; here, unlike with the 23S rRNA mutation, susceptibility to chloramphenicol and macrolides was also affected.¹⁵⁰

Recently, plasmid-mediated linezolid resistance has been identified. This results from production of the

Cfr methyltransferase, which gives cross-resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A compounds (the PhLOPS_A phenotype). The *cfr* gene was originally identified in staphylococci from animals,^{151–153} but there are now reports of it being found in human clinical isolates of *S. aureus* and *S. epidermidis*.^{154,155}

Daptomycin, a lipopeptide, was licensed for use in the United States in 2003 and in Europe in 2006. It has near-universal in vitro activity against *S. aureus* and is rapidly bactericidal.⁹² Of some concern is the positive correlation observed between decreased susceptibility to both vancomycin and daptomycin for clinical VISA/GISA isolates,¹⁵⁶ and for daptomycin-resistant mutants selected in vitro.¹⁵⁷ However, VISA are rare and most remain susceptible to daptomycin at the breakpoint. *Bona fide* resistance to daptomycin has emerged in isolates of MRSA during therapy resulting in therapeutic failure in endocarditis (rarely in other settings), with MICs rising from ≤ 0.5 mg/L to 2–4 mg/L.^{158–162} Even more marked daptomycin resistance has emerged in *E. faecium* during therapy, with MICs rising from 2 mg/L to ≥ 32 mg/L.¹⁶³ The mode of action of daptomycin and the mechanism(s) of resistance to it have not been completely defined,¹⁶⁴ though the compound is known to dissipate membrane potential and allow leakage of potassium ions.¹⁶⁵ Transferable daptomycin resistance has not been reported, but the compound is a natural product and producer strains of *Streptomyces roseosporus* must be self-resistant; they are a possible source of resistance genes.⁹² A high prevalence of strains able to inactivate daptomycin was reported in a study of antibiotic resistance among soil bacteria.¹⁶⁶

Glycylcycline (tigecycline) resistance has been observed in surveys. For example, in the BSAC Bacteraemia Surveillance Programme, tigecycline resistance (MICs > 0.5 mg/L) was noted in 0.4% of MRSA, in 2.8% of methicillin-susceptible coagulase-negative staphylococci, and in 10.9% of methicillin-resistant coagulase-negative staphylococci in the United Kingdom and Ireland.⁷¹ The emergence of resistance following prolonged tigecycline therapy has been reported in a single isolate of *E. faecalis* (MIC 2 mg/L, compared with the epidemiological cut-off value for susceptibility of ≤ 0.5 mg/L for the species) from Germany.¹⁶⁷ The mechanism of resistance in this isolate was not defined, but could not be transferred to other strains in vitro.

New fluoroquinolones, such as moxifloxacin, have good activity against pneumococci (far better than against staphylococci and enterococci), including against most isolates belonging to internationally distributed clones. However, even with pneumococci, we struggle to offset emerging quinolone resistance: Johnson et al. described the emergence of a fluoroquinolone-resistant strain of *S. pneumoniae* in England, with 48 isolates from nine centers.¹⁶⁸ Low-level and high-level resistant variants were reported with ciprofloxacin and moxifloxacin MICs of 8–32 and 0.5–1 mg/L for the former versus 64–256 and 4–16 mg/L for the latter. Representatives that were typed belonged to the Spain^{9V}-3 (ST156) clone, or were a novel ST (ST 609) related to it. Clonally related, levofloxacin-resistant pneumococci (MIC ≥ 8 mg/L) have also

been reported from Hong Kong, where they represent >15% of penicillin-resistant isolates.¹⁶⁹

Ketolides – telithromycin is the only licensed class member – are poor substrates for macrolide efflux pumps, and remain active also against pneumococci with Erm rRNA methylases. Data from the PROTEKT surveillance continue to show a very low prevalence (<1%) of telithromycin resistance in pneumococci.^{110,111,170} Most of the few resistant isolates have low-level resistance (MICs 4–8 mg/L) and represent diverse clonal types. However, the emergence of high-level ketolide resistance (the MIC rose from 0.12 to 256 mg/L) was reported in a highly fluoroquinolone-resistant pneumococcus (moxifloxacin MICs 8–16 mg/L) following two 10-day courses of telithromycin. The pre-therapy isolate was resistant to macrolides and carried a *mef(A)* determinant, as did the post-therapy isolate. In addition, however, the post-therapy isolate had a mutation of residue A2058 of the 23S rRNA and a three amino acid deletion in ribosomal protein L22. These changes conferred resistance not only to telithromycin, but also to clindamycin, which was active against the pre-therapy isolate. Rantala et al. have reported heterogeneous resistance to telithromycin in pneumococci carrying an *ermB* determinant.¹⁷¹

6. Conclusions

We have at our disposal a range of molecular tools that allow us to dissect the population structure and epidemiology of Gram-positive pathogens. The species of most concern have not changed in the last two decades, but they have become more resistant. The biggest problem is still MRSA, which is hugely clonal and becoming more problematic, with new highly toxigenic community strains emerging globally, and with the possibility that they will supplant established health care associated clones, even in hospitals. Vancomycin-resistant enterococci are also widespread, but are a greater problem in the United States than in Europe. Many of the strains that cause hospital outbreaks in different countries represent expansion of a single successful clonal lineage by direct spread or repeated selection in multiple places. In addition, the horizontal spread of glycopeptide resistance genes is superimposed on this background.

We can also track the international spread of successful clones of pneumococci and monitor their divergence over time. In some countries, such as the United Kingdom, we are seeing a decline in the prevalence of penicillin-resistant strains, although there is little evidence of any comparable decreases in rates of macrolide resistance. Now 11- and 13-valent conjugate anti-pneumococcal vaccines are already in advanced development and the latter covers the emerging penicillin-resistant serotype 19A strain.

Gram-positive bacteria other than this traditional triumvirate have also emerged as serious public health issues. *Clostridium difficile* has long been recognized as the cause of a range of gastrointestinal disease in hospitalized elderly patients.¹⁷² Disturbingly, a new epidemic strain, defined as North American PFGE type 1 and PCR-ribotype 027 (NAP1/027), has recently emerged and is associated with increased severity, high relapse

rate, and significant mortality.^{173,174} It is also resistant to fluoroquinolones,^{175–177} and may be selected by them; more generally, the selection of *C. difficile* has been associated with overuse of cephalosporins.

For the future, we need to maximize the effectiveness of intervention methods to reduce the burden of disease and economic consequences of infections caused by Gram-positive bacteria. We need to determine urgently whether we can control and reduce MRSA rates in those hospitals and countries where it is already endemic at high levels. Recent declining rates of MRSA bacteremias in the United Kingdom are encouraging, but need to be maintained in the longer term. Our efforts are constantly threatened by continuing resistance development. Currently, this is represented by low numbers of staphylococci and enterococci resistant to linezolid or to daptomycin, and by pneumococci resistant to fluoroquinolones or ketolides. Prompt recognition of isolates resistant to these newer agents is of paramount importance, and will allow appropriate treatment of affected patients and implementation of infection control procedures. We must strive to keep the prevalence of such resistant strains low.

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