ELSEVIER

Microbiology and drug resistance mechanisms of fully resistant pathogens

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The acquisition of vancomycin resistance by Gram-positive bacteria and carbapenem resistance by Gram-negative bacteria has rendered some hospital-acquired pathogens impossible to treat. The resistance mechanisms employed are sophisticated and very difficult to overcome. Unless alternative treatment regimes are initiated soon, our inability to treat totally resistant bacteria will halt other developments in medicine. In the community, Gram-positive bacteria responsible for pneumonia could become totally resistant leading to increased mortality from this common infection, which would have a more immediate impact on our current lifestyles.

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Abbreviations

MIC	minimum inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
VRE	vancomycin-resistant *Enterococcus faecium* and *faecalis*

Introduction

Antibiotic resistance is reaching a crisis because we are running out of options to treat certain pathogenic bacteria, mainly causing hospital-acquired infection but with the potential to occur in the community. These multi-resistant bacteria are generally identified by the acquisition of specific resistance genes that are catastrophic. The development of these resistant strains has built up progressively over the past 20 years but has come to a head within the past five. Although there were incidences of outbreaks of multi-resistant bacteria in the past 10 years, these are now becoming more frequent and more persistent. The incidence of resistant bacteria used to fall back a little after intervention but now this occurs less readily, largely because we do not have effective antibiotics to treat them. The extrapolation of fewer periods of remission from multi-drug resistance is, of course, that these bacteria progress to pan-resistance where no class of antibiotics can be used effectively to treat them. This is the situation we are beginning to experience in some clinical bacteria.

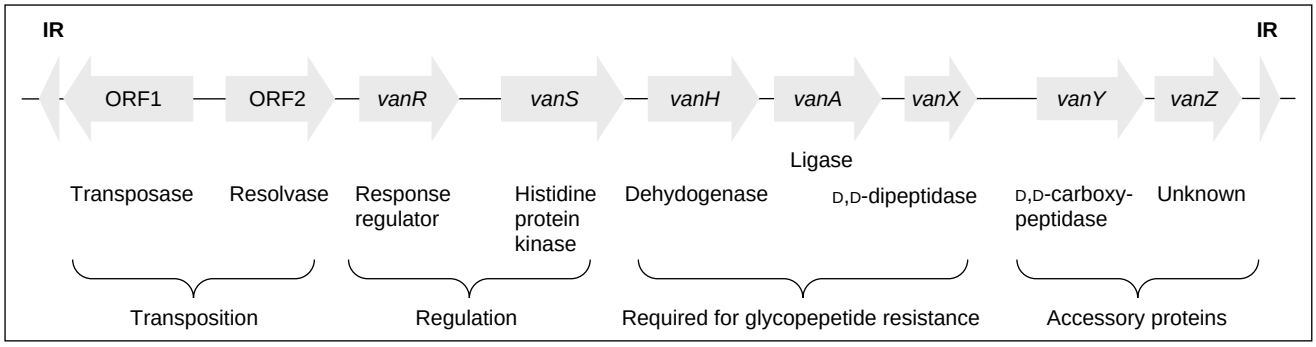
In this review, we focus on those bacteria where pan-resistance has been or is set to be a problem.

Hospital-acquired infections

Some hospital-acquired pathogens are becoming totally resistant to antibiotics, known examples include vancomycin-resistant *Enterococcus faecium* and *faecalis* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [1]. The failure is often attributed to the misuse of antibiotics but the truth is probably that medicine has moved on and too much is now expected from antibiotics especially in the treatment of the immunocompromised. These patients are a fertile breeding environment for resistant bacteria, illustrated by the rise in Acinetobacter and VRE infections in intensive care units, which is largely attributed to infections in immunocompromised patients; such pathogens are not considered particularly pathogenic elsewhere.

Prior to the introduction of linezolid and synercid, VRE were the first hospital bacteria to become resistant to all available antibiotics. These organisms, often identified as commensals of the human gastrointestinal tract, were found to be the second most prevalent pathogen in intensive care units in the United States. The vancomycin resistance was initially identified to be conferred by an operon of eight genes (Figure 1). Vancomycin resistance is known to be transferable [2] and many transferable resistance mechanisms are the result of single genes; vancomycin resistance thus showed a quantum leap in the sophistication of resistance determinants. The original operon was bound by insertion sequences encoding transposon Tn*1546* [3]. The two genes of a classic class II transposon ensure the migration of the gene between replicons and different strains. Three essential genes (*vanH*, *vanA* and *vanX*) are responsible for initiating an alternative route to peptidoglycan synthesis that removes the glycopeptide binding site of the D-alanyl-D-alanine group on the pentapeptide. This alternative route involves reversing the formation of the D-alanyl-D-alanine moiety (VanX) to release free D-alanine, hydrogenating pyruvate to form lactate (VanH), which is then ligated with the freed D-alanine to provide D-alanyl-lactate

Figure 1

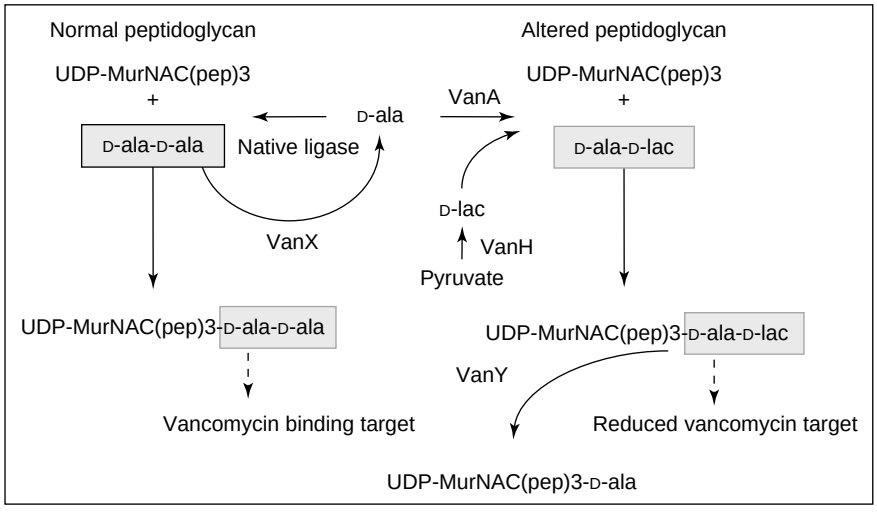


The *vanA* operon [3].

(VanA). This moiety is now incorporated in the normal peptidoglycan synthesis and is no longer susceptible to glycopeptide inhibition. On cross-linking during transpeptidation, the terminal lactate is removed, often by the non-essential gene product VanY [4] (Figure 2). An additional non-essential gene (*vanZ*) has no known function. The *vanA* operon is inducible, which is also rare for a transferable resistance mechanism, by a two-component induction system. VanS is a transmembrane protein that autophosphorylates when it detects an incoming glycopeptide, which then phosphylates VanR that acts on the essential promoter of the operon. The *vanA* operon is effective against all glycopeptides that can initiate induction including teicoplanin. There are several similar operons in VRE; the second in importance is the *vanB* operon in Tn*1547*, which confers lower resistance to vancomycin and none to teicoplanin because this glycopeptide does not act as an inducer [5]. The *vanC* and *vanE* operons employ serine instead of lactate as the glycopeptide-insusceptible terminal moiety in the pentapeptide in peptidoglycan synthesis [4,6].

Variations in the *vanA* operon that are dependent on the area that the host pathogen has been isolated have been identified; for instance, the nucleotide sequence of operons from strains isolated in the United States differs from those in the UK [7]. However, this operon can also vary within a small geographical area and different insertions

Figure 2



The mechanism of vancomycin resistance conferred by the *vanA* operon [3]. This diagram shows the mechanism of action of the *vanA* operon. On the left hand side is the pathway for normal peptidoglycan synthesis, where two D-alanine residues are ligated as a precursor. The *vanA* operon encodes a D,D-dipeptidase (VanX) that reverses this reaction to provide D-alanine. The dehydrogenase VanH converts pyruvate to lactate that, under the action of the VanA ligase (VanA), forms D-alanyl-D-lactate. This becomes the new precursor and is incorporated into peptidioglycan. Unlike D-alanyl-D-alanine, D-alanyl-D-lactate does not readily bind vancomycin and thus provides resistance to it. The D-lactate is lost in transpeptidation so the final composition of the peptidoglycan is the same. The *vanA* operon also encodes a D,D-carboxypeptidase, but this gene is not essential.

were found upstream of the main promoters in strains isolated from different health regions of Scotland; these insertions have been linked to the capability to generate teicoplanin resistance and may mirror teicoplanin policies within individual health regions [8•].

The proportion of *S. aureus* infections that are methicillin-resistant in hospitals in the UK has risen alarmingly by nearly 20-fold in the second half of the 1990s to just under 40% [9]. The spread of MRSA is known to be clonal in the UK; there are two main types, EMRSA-15 and EMRSA-16, first identified by phage typing [10]. These account for more than 95% of all MRSA isolated. The integrity of the types has been verified by genotyping. Six strains of *S. aureus* have been sequenced and there is conservation in 78% of the genome with considerable diversity in the remaining *22%*; however the main epidemic MRSA strains do show some common characteristics in that they all carry the *seg* and *sei* enterotoxin genes but, unlike less common MRSA, do not carry the *lukE* leucocidin and *splB* serine protease genes [10]. The use of methicillin resistance as a marker is misleading; *S. aureus* acquired resistance to this penicillin in the 1960s. It was the acquisition of aminoglycoside resistance with methicillin resistance that generated treatment problems; however the bacteria could still be treated with glycopeptides. In the mid 1990s, Hiramatsu identified strains of MRSA isolated in Japan that had intermediate susceptibility to vancomycin, with minimum inhibitory concentrations (MICs) of 8mg/L [11]. Hiramatsu also demonstrated that strains from the same area that were vancomycin susceptible could become intermediate at relative high frequency [12]. Further investigation revealed that the bacteria had increased their cell wall production and now had a much thicker cell wall than the parent strains, which provided some barrier to vancomycin [13]. Infections caused by these strains, now known as VISA (vancomycin-intermediate *Staphylococcus aureus*) could still be treated. An *in vitro* experiment 10 years ago demonstrated that the much more efficient vancomycin resistance mechanism of the *vanA* operon from VRE could transfer and survive in MRSA [14••]; fortunately this was not found in clinical strains. The situation changed in 2002 when the first MRSA strain harbouring a *vanA* operon was found in a patient in Michigan [15••]. This was followed by reports of a strain in Pennsylvania [16,17] and later in New York. Courvalin recently described at the 14th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Prague that the *vanA* operon is very similar to that in VRE but that the mobile element on which it is located is not stable in *S. aureus*. To survive, Tn*1546* had to have sufficient opportunity to 'jump' into a more stable replicon in *S. aureus*. The rarity of reports of vancomycin-resistant *S. aureus* strains around the world, despite the pressure that there must be to select them, suggests that this operon has yet to find a sufficiently stable genetic environment in MRSA, but surely it will.

Despite the attention focussed on VRE and MRSA, Gram-negative bacteria were the first pathogens responsible for hospital-acquired infections. The most problematic of these when ability to treat and pathogenicity are considered are *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Both these non-fermentors have few porins, small in the case of *A. baumannii*, which have given them a predisposition to survive in an antibiotic-rich environment. They are responsible for most hospital-acquired pneumonia in patients previously treated with antibiotics [18] as well as causing many other life-threatening infections. Their ability to rapidly acquire resistance to cephalosporins and fluoroquinolones has left the carbapenems as the only remaining viable treatment option. Both species can become resistant to carbapenems by genetic mutation, to hyperproduction of the class C chromosomal β-lactamase in *P. aeruginosa* increasing the number of active sites able to bind carbapenems even if they are inefficient at hydrolysing them or by loss of porins in *A. baumannii*. However, it is their capability to accept transferable β-lactamase genes that confer carbapenem resistance at a level that will result in clinical failure that causes most concern. In the mid 1990s, *P. aeruginosa* strains isolated in Japan were found to harbour a transferable class B metallo-β-lactamase called IMP-1 [19]. This enzyme could increase the MIC to greater than 128mg/L depending on the permeability characteristics of the strain. This gene was thought originally to be confined to southeast Asia but it and a myriad of similar genes ($bla_{\text{IMP-2}}$ – $bla_{\text{IMP-12}}$) have now been isolated in *P. aeruginosa* and other Gram-negative bacteria from around the world. A similar class of β-lactamases (VIM 1–4) have been found in Asia and Europe, almost exclusively in *P. aeruginosa* [20]. The extent and diversity of these enzymes suggests that they will be very difficult to overcome.

Although the bla_{IMP} genes can migrate into *A. baumannii*, they do not appear to be a major contributor to carbapenem resistance. A new group of transferable class D β-lactamases have emerged almost exclusively in *A. baumannii* and are capable of increasing the MIC of imipenem to 16mg/L or higher. The first of these enzymes (OXA-23) was found in Scotland 15 years ago [21••]. This enzyme was transferable [22] and had a unique amino acid sequence [23•]. Thought to be an isolated observation, $bla_{\text{OXA-23}}$ has subsequently been found in Brazil [24] and very recently has been reported to be responsible for two outbreaks in southeast England. A second group of class D carbapenemases has been found in *A. baumannii* isolated largely in southwest Europe, these enzymes share about 60% homology with OXA-23 [25]. The success of the vancomycin-resistance operon in Gram-positive pathogens and transferable carbapenamses in Gram-negative pathogens is certain to make treatment of hospital infection very difficult or near impossible. The introduction of linezolid and the streptogrammins might

temporarily alleviate the situation in Gram-positive bacteria although problems have already been identified, but the continuing infiltration in Gram-negative bacteria by carbapenamases is likely to render these bacteria impossible to control as has already been experienced in some parts of the world [26].

Among community-acquired infections in the developed world, those caused by *Streptococcus pneumoniae* are the most likely to develop resistance progressively to the major groups of available antibiotics. In general, the resistance most associated with *S. pneumoniae* is penicillin resistance. However, penicillin resistance has previously included intermediate resistance and thus falsely elevated the levels of penicillin resistance reported worldwide. Currently, penicillin resistance in *S. pneumoniae* varies from 0% resistance in the Netherlands to 71.5% in South Korea [27]. Although this is problematic, penicillin resistance is at least concentrated in only certain countries, whereas the levels of resistance to macrolides are both high and widespread throughout the world. In many countries macrolide resistance is higher than penicillin resistance and does not appear to be arresting. One such country is Hungary, where penicillin resistance is 2% (37% intermediate) but erythromycin resistance is 41.7% [28], another is Italy with penicillin resistance at 10.1% but erythromycin resistance at 42.9% [27].

The most frequently identified macrolide resistance mechanisms are methylation of the target site associated with the *erm*B gene or efflux encoded by the *mef* genes. These two mechanisms have been identified worldwide, where testing has been performed. However, distribution of these genes has been suggested to be continent dependent. Macrolide resistance in the USA and Canada is predominantly mediated by the *mef* genes and so has the M phenotype. While in Europe, macrolide resistance is mostly associated with the presence of the *erm*B gene and the MLS_B phenotype. Recently, the resistance profile of Europe has begun to change as resistance data has been collected from countries other than those with a high level of macrolide resistance. The most recent publications currently indicate that within Europe resistance to macrolides due to the *mef* gene is increasing, whereas resistance due to the *erm*B gene is remaining static. This is happening both in countries with high macrolide resistance, such as Italy [29] and low macrolide resistance such as Finland [30]. The efflux phenotype is the predominant macrolide resistance mechanism in the UK, Sweden, Finland, Germany, Switzerland and Austria [31,32]. Are these isolates or their genes spreading to the rest of Europe? The total European picture is unclear but there are trends emerging that the *mef* gene mediated resistance is beginning to take over the *erm*B-based resistance. If this is the case then we need to know how this is happening and why. This is of most importance in relation to the new class of antibiotics, the ketolides. Telithromycin has excellent activity against *erm*B and *mef* mediated macrolide resistances [27]. Mutation studies have identified that *S. pneumoniae* resistant to telithromycin have occurred with fewer passages or generations in *S. pneumoniae* with the *erm*B gene than those containing the *mef* genes [33,34]. While the increasing presence of the *mef* macrolide resistance augurs well for the ketolides, we do need more research into the effects of this gene and the effect of efflux on the new ketolide class.

The results of genotyping studies on *S. pneumoniae* have identified a group of 16 multi-drug resistant clones circulating the world [35]. Resistance to macrolides is usually mediated by genes on mobile elements. The relationship between macrolide resistant isolates is not clonal but depends on the movement of resistance genes between isolates [36]. Penicillin resistance is not mediated by mobile elements. Therefore, macrolide resistance is spread by gene movement and penicillin resistance is spread by isolate movement. Thus, as gene movement is a more frequent event than isolate movement the ability for macrolide resistance to spread is of higher concern than penicillin resistance spread. We believe that the increase in macrolide resistance as opposed to penicillin resistance is associated with the ability of the macrolide resistance genes to transfer more efficiently than the penicillin resistant isolates to disseminate.

In recent years, research into macrolide resistance has dramatically increased both in the number of isolates studied and the number of countries involved in surveillance of macrolide resistance in *S. pneumoniae*. Studies of macrolide resistance and in particular surveillance studies have not only focused on the levels of macrolide resistance but have also investigated the mechanisms of resistance used by the isolates in each country. The increased level of research both within clinical isolates and *in vitro* has provided us with a greater knowledge of the mechanisms of macrolide resistance and particularly the less frequently identified resistance mechanisms. The mechanisms of macrolide-resistance in *S. pneumoniae* were initially confined to the identification of either the *erm* or *mef* genes. The study of macrolide resistance was then expanded to include macrolide resistant *S. pneumoniae*, which did not harbour either the *erm*B or *mef*A genes. This included investigation of the 23S rRNA alleles and the L4 and L22 riboproteins for mutations [37]. Following the publication of this paper, isolates proving negative for the *erm*B or *mef* genes were routinely analysed for mutations in these regions. While resistance is predominated by the *erm*B or *mef* genes these mutations do also occur in clinical isolates. As research moved outside of the *erm*B and *mef* genes further discoveries were made: there are two types of *mef* gene, *mef*A and *mef*E carried by two different transposons, the presence of an

*erm*A gene in *S. pneumoniae*, which confers macrolide resistance and isolates containing both *erm*B and *mef* genes were also identified [38–40].

What are the antibiotics of last resort for the treatment of macrolide resistant, penicillin and multi-drug resistant *S. pneumoniae*? Currently, they are linezolid and vancomycin. Unlike *S. aureus* and *enterococci* vancomycin-resistant *S. pneumoniae* have not yet been identified. However, clinical strains of vancomycin tolerant *S. pneumoniae* have been identified [41,42]. Tolerance is the ability of bacteria to survive in the presence of an antibiotic, neither growing nor undergoing lysis. It is thought to be a precursor to resistance development. As vancomycin tolerance is not routinely documented in the surveillance studies as with macrolide resistance it is difficult to identify the full extent of vancomycin tolerance.

Conclusions

Antibiotic resistance in these pathogens is set to change the way we view medicine; elective surgery with the risk of an untreatable infection is an unattractive option and transplantation with the likelihood that any infection may lead inevitably to death is a gamble that many may not be prepared to take. However, total resistance in the community is likely to have a more immediate impact on the way we live, few parents would be prepared to send their children to day-care centres if the danger of untreatable infection is high.

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