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Current and emerging issues in nosocomial infections and antibiotic resistance

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Abstract

Antibiotic resistant pathogens are a major cause of nosocomial infections and exhibit an extraordinary ability to constantly adapt and acquire resistance determinants to overcome the effects of commonly prescribed antimicrobials. Glycopeptide resistant *Enterococcus faecium*, multidrug resistant *Pseudomonas aeruginosa*, *Clostridium difficile* and *Escherichia coli* are all pathogens of clinical interest, increasing in prevalence and causing large outbreaks of infection within hospitals. On the other hand, the emerging potential of vancomycin resistant *Staphylococcus aureus* (VRSA) and linezolid resistance in Gram-positive pathogens to follow suite provides a serious concern for the future treatment of hospital-acquired infections.

Keywords: Antibiotic resistance, nosocomial infection, vancomycin, multidrug resistance, linezolid

Introduction

Antibiotic resistant bacteria are a major cause of nosocomial infections and are associated with increasing rates of mortality among hospitalised patients (Rossolini *et al*., 2010). The ability of these microorganisms to adapt and overcome the effects of therapeutic drugs provides an ever changing clinical battle. Of particular concern is the spectrum of antibiotic resistance exhibited by certain bacteria as new antimicrobials cannot be synthesised or discovered quickly enough. The resistant bacteria of current clinical interest are *Staphylococcus aureus, Enterococcus faecium, Clostridium difficile, Pseudomonas aeruginosa* and *Escherichia coli*. These pathogens are able to evade the effects of antibiotics via a multitude of mechanisms compromising the effective treatment of infections (Table 1).

The hospital environment provides great selective antimicrobial pressure and a suitable area for dissemination of resistance determinants; the use of antibiotics and the presence of sensitive microorganisms allow the acquisition and transfer of resistance genes and thus the emergence of highly pathogenic bacteria (French, 2010). The shift from sensitive to resistant bacteria populations is considered as an inevitable evolutionary response due to the extensive and concentrated use of antibiotics, making the future of successful treatment indeterminate. This article aims to review the current literature on the prevalence and mechanisms of antibiotic resistant nosocomial pathogens and consider the impact of this on the treatment of infections caused by such bacteria.

Table 1: Nosocomial pathogens, resistance phenotype and mechanisms (adapted from Rice, 2009; Rossolini et al., 2010)

Species	Resistance Phenotype	Mechanism(s)
Staphylococcus aureus	Methicillin	Altered penicillin-binding proteins (PBPs)
	Penicillin	β-lactamase
	Oxacillin	Low-affinity PBP
	Clindamycin	Constitutive erm expression
	Vancomycin	Mechanism unclear
Enterococcus faecium	Ampicillin	Low affinity PBPs
	Vancomycin	Altered peptidoglycan precursor
	Linezolid	Mutant ribosomal RNA genes
	Daptomycin	Mechanism unclear
Clostridium difficile	Rifamycins	Mutations in rpoB gene (decrease affinity of RNA target)
	Macrolides & Clindamycin	Ribosomal target methylation by Erm expression
	Fluoroquinolones	Mutant DNA gyrase subunit
Pseudomonas aeruginosa	Carbapenems	Metallo-β-lactamases AmpC/porin reduction combinations
	Aminoglycosides	Modifying enzymes
	Fluoroquinolones	Mutant topoisomerases Efflux pumps
	Penicillins	β-lactamases
Escherichia coli	Penicillins	β-lactamases
	Fluoroquinolones	Efflux pump
	Aminoglycosides	Aceyltransferases

Vancomycin Resistant *Staphylococcus aureus*

Staphylococcus aureus is undoubtedly the most notorious and prevalent Gram-positive nosocomial pathogen found in clinical samples, being a leading cause of both skin structure infections and blood stream infections (EARRS, 2010). The ability of this microorganism to respond to its environment and adapt accordingly has enabled it to become resistant to an array of antimicrobials, thus providing a major resistance challenge within the health-care setting. Vancomycin, a glycopeptide antibiotic, was first used clinically in 1958; however, its use has dramatically increased in the last thirty years as an effective treatment of severe methicillin resistant *S. aureus* (MRSA) infections (Srinivason *et al*., 2002). For this reason, the emergence of vancomycin intermediate *S. aureus* (VISA) and vancomycin resistant *S. aureus* (VRSA) strains is incredibly alarming.

VISA was first displayed clinically in Japan in 1996, and although relatively uncommon the potential clinical challenge it poses should not be underestimated (Hiramatsu et al. 1997). This phenotype exhibits a thickened and poorly cross-linked peptidoglycan layer, able to sequester vancomycin. Cui *et al.* (2006) identified that the altered structure of the peptidoglycan has an increased number of D-ala-D-ala targets able to bind to the antibiotic within the outer extremities of the cell wall, thus sequestering it. However, Rossolini *et al.* (2010) emphasise the importance of the increased thickness of the peptidogylcan in preventing vancomycin from accessing and binding to the D-ala-D-ala targets. On the other hand, Periera *et al*. (2007) highlighted the decrease in the rate of diffusion of vancomycin to its target as a key consequence of the thickened cell wall. Although not in agreement on the exact mechanism, these studies express the vital role of the modified peptidoglycan in preventing vancomycin from reaching the cytoplasmic membrane where it can exert its bactericidal effect. They also present the possibility that resistance and reduced susceptibility to vancomycin may indeed be multifactorial.

VISA are mediated by chromosomal mutations; gradual mutations within sensitive strains of *S. aureus* involving such loci as vraSR and graSR lead to heterogenous VISA (hVISA), which in turn develop subpopulations of homogenous VISA (Howden *et al*., 2010). This is regulated by a two component signal-transduction system (Van RS in the case of vancomycin resistance). VanRS is activated in the presence of vancomycin, thus activating the specific loci (vraSR and graSR) and ultimately stimulating a defence mechanism (Neoh *et al.*, 2008; Depardieu *et al,* 2007). Although beneficial, these multiple genetic alterations are associated with high fitness costs. Slower growth, decreased expression of virulence factors and reduced stability has been exhibited by VISA in vancomycin free medium (Majcherczyk *et al.,* 2008; McAleese *et al.,* 2006). It can be proposed that these biological fitness issues are the reason for the current low prevalence of VISA in hospitals.

More recently, and of greater concern is the emergence of high-level VRSA via the acquisition of van gene complexes from enterococcus species. Since 2002 eleven clinical VRSA isolates possessing the vanA operon have been reported (French, 2010). The vanA operon is carried by the transposon Tn1546 and can be transferred to *S. aureus* by one or two genetic events. The initial step is the transfer of Tn1546 from enterococci to *S. aureus* by conjugation. As some enterococcal plasmids are less efficient at replication and unstable in *S. aureus* a further step is required; transposition of Tn1546 from the donor plasmid to a plasmid or chromosome located in *S. aureus* results in the illegitimate recombination of Tn1546 and the loss of the

enterococcal plasmid (Zhu *et al.,* 2008). It is this operon that mediates the synthesis of altered peptidoglycan precursors (D-ala-D-lactate or D-ala-D-serine depsipeptides) and the production of D,D-peptidases that hydrolyse any D-ala-D-ala terminating peptidoglycan precursors, thus preventing vancomycin from reaching its target and ultimately conferring glycopeptide resistance to *S. aureus* (Perichon *et al.*, 2009). Although Rossolini *et al.,* (2010) argues VRSA is not expected to play a major role as a nosocomial pathogen in the near future, Perichon *et al.,* (2009) states the acquisition of vancomycin resistance by *S. aureus* is a 'major public health problem'. Indeed the prevalence of MRSA and enterococci, along with the increased use of vancomycin in hospitals provides great potential for VRSA to consistently form and disperse within a clinical setting.

The continued ability of *S. aureus* to become resistant to virtually all antibiotics is evident in the development of linezolid resistance. Linezolid is the new 'last line drug' for the treatment of *S. aureus* infections, however in 2008 a clinical outbreak of linezolid resistant *S. aureus* (LRSA) was observed in an intensive care unit (Fereres *et al.*, 2010). This outbreak was the first to be mediated by the cfr gene, (confers chloramphenicol-florfenicol resistance) which is a transferable mechanism and therefore of considerable concern (especially as linezolid is fully synthetic). The cfr gene encodes a methyltransferase that catalyses the methylation of A2503 in the 23S rRNA gene of the large ribosomal subunit, thus altering the antibiotics target and preventing it from inhibition (Morales et al. 2010). An alternative mechanism of LRSA is associated with chromosomal point mutations in the drug's target site (V region of 23S rRNA) induced by prolonged exposure to the antibiotic, although this remains uncommon it does identify the generation of de novo resistance (Besier *et al.,* 2008).

Glycopeptide resistance in Enterococci

Enterococci are Gram-positive bacteria that typically colonise the gastrointestinal tract of humans and animals. Once thought to be harmless commensals, enterococci have emerged as a key nosocomial pathogen causing urinary tract infections, bloodstream and surgical site infections in hospitalised patients (Rossolini *et al.,* 2010; Top *et al.*, 2008). Intrinsically resistant to a number of antibiotics and able to easily acquire resistant plasmids (Table 2), *Enterococcus faecalis and E. faecium* are the two species of particular clinical interest.

Vancomycin resistant enterococci (VRE) were first identified in Europe in 1986 and the prevalence of infections have been increasing since 2000 (Uttley *et al.,* 1988; EARSS, 2010). The majority of clinical infections (80-90%) were caused by *E. faecalis* (Low *et al.*, 2001). However over the past few years *E. faecium* has emerged as a significant multi-resistant nosocomial pathogen due to its ability to adapt and exploit the hospital environment, and readily exchange antimicrobial resistant genes (Top *et al.,* 2008). Indeed Willems *et al.,* (2005) identified that most of the hospital-acquired VRE infections are caused by a single genetic lineage of *E. faecium*, clonal complex 17 (CC17). CC17 is a prime example of how stress inducing factors (antimicrobial use within hospitals) can favour the selection of a subpopulation exhibiting enhanced virulence and ability to spread due to the acquisition of antimicrobial resistance. The resilience of enterococci to survive rigorous cleaning and sterilisation in a clinical setting has also undoubtedly contributed to its establishment as one of the most formidable nosocomial pathogens (Kearns *et al.,* 1995; Hayden, 2000). It has been suggested that the emergence of VRE was due, directly, to an increased use of vancomycin in the USA, thus

highlighting the adverse consequence of using antibiotics (Bonten *et al.,* 2001). However Witte (2004) draws attention to the central role of *E. faecium* in acquiring, conserving and transferring antibiotic resistance genes.

Table 2: Antibiotic resistance of enterococci (adapted from Top et al. 2008)

	Antibiotic	Species	Resistance Mechanism
Intrinsic resistance	β-Lactams Penicillins (low level) Carbepenems (moderate level) Cephalosporins (high level)	All enterococci	Low affinity PBPs
	Aminoglycosides (low level)	All enterococci	Inefficient uptake
	Aminoglycosides (moderate level)	*E. faecium*	Production of chromosomal AAC(6')li enzyme
	Lincosamides & streptogramins A	*E. faecalis, E. avium, E. gallinarum, E. casseliflavus*	Putative efflux
	Glycopeptides (low level)	*E. gallinarum, E. casseliflavus*	Production of D-Ala-D-Ser peptidoglycan precursors
Acquired resistance	Ampicillin (high level)	*E. faecium, E. hirae E. faecalis*	Overproduction or alterations of PBP5 B-lactamase (rare)
	Aminoglycosides (high level)	*E. faecalis, E. faecium, E. gallinarum, E. casseliflavus*	Aminoglycosides modifying enzymes e.g. AAC (6')-APH (2")
	Macrolides	Most enterococci	Ribosomal methylation
	Chloramphenicol	*E. faecium, E. faecalis*	CAT encoding enzymes
	Tetracycline	*E. faecium, E. faecalis*	Modification of ribosome protein
	Quinolones	*E. faecium, E. faecalis*	Alterations in DNA gyrase and Topoisomerase IV
	Glycopeptides (high level)	*E. faecium, E. faecalis*	Peptidoglycan precursor modification
	Oxazolidinones	*E. faecium*	Mutation in 23S rRNA gene

Vancomycin resistance is mediated by the acquisition of van gene clusters which code for the production of modified peptidoglycan. The D-ala-D-ala dipeptide is replaced by a depsipeptide composed of D-alanyl-D-lactate or D-alanyl-D-serine, thus lowering the affinity of the glycopeptide-binding target (Reynolds and Courvalin, 2005; Boyd *et al.*, 2008). Numerous van gene clusters have been identified in enterococci (vanA to vanL), with an eighth recently described by Xu *et al.,* (2010); vanM is considered to confer vancomycin resistance via a similar mechanism to that of vanA, (inducible synthesis of D-alanyl-D-lactate ligase and able to be transferred by conjugation), however identification of this van cluster remains uncommon at present. VanA and vanB are the most prevalent van gene clusters in clinical isolates and are located on transposons; Tn1546 and Tn1549 respectively (Rossolini *et al.,* 2010). As Tn1546 does not encode any conjugative functions, but Tn1549 does, it can be inferred that dissemination of vancomycin resistance is due to both the clonal expansion of resistant strains and the horizontal gene transfer between strains (Top *et al.,* 2008). These mobile genetic elements have played a huge part in the development of highly hospital-adapted strains and are now moving glycopeptide

resistance to other bacterial species such as *S. aureus* (as previously described)which causes great concern when vancomycin is referred to as a 'last line' drug.

Linezolid is a key therapy for the treatment of VRE, and it was thought that development of resistance would be a rare event. However, in recent years linezolid resistance has been exhibited both in vitro and in clinical isolates of enterococci (Gomez-Gil *et al.,* 2009; Gonzales *et al.,* 2001). Similarly to *S. aureus*, the main mechanism of resistance is attributed to the single point mutation G2576U within the 23S rRNA gene, which alters the target of the drug (Scheetz *et al*., 2006). Furthermore, a recent study by Long et al. (2010) identified that double 23S rRNA mutations have a remarkable synergistic effect on linezolid resistance, in comparison to the effects of the corresponding single mutations. Although surveillance programmes stress it is still uncommon in hospitals, research has identified the formation of linezolid-resistant subpopulations suggesting the continued emergence of this phenotype upon increased use of linezolid (Farrell *et al*., 2009; Allen *et al.,* 2009).

Multidrug Resistant *Clostridium difficile*

Clostridium difficile, a Gram- positive bacterial bacillus, differs from other nosocomial pathogens in that the emergence and prevalence of resistant strains is not directly linked to the treatment of *C. difficile* infection (CDI). Resistance to erythromycin, clindamycin and fluoroquinolones is exhibited among most pathogenic strains of *C. difficile* and is considered to have arisen due to their use in the treatment of other bacterial infections (Huang *et al.*, 2009). The use of broad-spectrum antibiotics alters the microflora of the intestinal gut (where *C. difficile* colonise) thus allowing pathogenic strains of *C. difficile* to proliferate and CDI to occur. Many consider antibiotic usage and poor hygiene within a clinical setting as key underlying factors of CDI outbreaks (French, 2010).

Large outbreaks of CDI have been found to be caused by so-called 'hypervirulent' strains, such as polymerase chain reaction (PCR) ribotype 001, 027 and 106. PCR ribotype 027 is considered as the most common in clinical isolates; indeed studies have shown these strains to be associated with an increased severity of infection and high mortality rate (Coia, 2009). Features of all PCR ribotypes include deletions in the tcdC regulatory locus allowing the hyperproduction of toxins A and B, and resistance to an array of antibiotics (particularly fluoroquinolones) (McDonald *et al.,* 2005). The resistant mechanisms exploited by *C. difficile* vary depending on the agent used; ribosomal methylation mediated by erm genes confers high level resistance to erythromycin and clindamycin, whereas fluoroquinolone resistance is caused by amino acid substitutions in the quinolone-resistant determining region (QRDR) of target enzymes (typically GyrA or GyrB subunits). In addition, an increased efflux of the drug has been identified to confer fluoroquinolone resistance (Huang *et al.*, 2009).

Vancomycin and metronidazole are the antibiotics of choice to treat CDI. Although resistance to these agents remains uncommon, a decreased susceptibility has been reported in several studies. Pelaez *et al.,* (2008) illustrated that metronidazole resistance is inducible and Baines *et al.*, (2008) described an increased minimum inhibitory concentration (MIC) for metronidazole for PCR ribotype 001 in the UK. Although the exact mechanisms remain to be elucidated, the reduced uptake of

metronidazole and reduced nitroreductase activity have been suggested as possible methods. Similarly, Mutlu *et al.,* (2007) demonstrated an increase in the number of isolates displaying an increased MIC for vancomycin. Again, the mechanism of resistance remains unclear, however the acquisition of van gene clusters from enterococci could be possible as Jasni *et al.*, (2010) demonstrated that the transfer of resistance genes between the two bacteria can occur.

Multidrug Resistant *Pseudomonas aeruginosa*

Pseudomonas aeruginosa, a Gram-negative rod shaped bacterium, is a major cause of nosocomial respiratory, urinary and bloodstream infections in immunocompromised patients, especially those suffering with cystic fibrosis. Antibiotic resistance is a key factor in the pathogenicity of this microorganism, as infection caused by resistant strains is associated with a threefold increased rate of mortality (Mesaros *et al.*, 2007). *P. aeruginosa* provides a particularly problematic clinical challenge as not only is it intrinsically resistant to an array of antibiotics, its high versatility and adaptive capacity allows it to acquire resistance traits as well. Furthermore, its ability to form biofilms on medical equipment (catheters and prosthetic joints) and the lungs of cystic fibrosis patients makes effective treatment rather complicated, and sometimes near impossible.

Planktonic *P. aeruginosa*, as with other nosocomial pathogens, display a multitude of mechanisms to overcome the bactericidal and bacteriostatic effects of antibiotics (Table 3).

Table 3: Mechanisms of resistance exhibited by *Pseudomonas aeruginosa* (adapted from Mesaros et al. 2007)

Antibiotic	Mechanism of resistance
Penicillins and cephalosporins	Active efflux Cephalosporinase overexpression Restricted-spectrum penicillinases Extended-spectrum oxacillinases Extended-spectrum β-lactamases Metallo- β-lactamases
Aztreonam	Active efflux Cephalosporinase overexpression Restricted-spectrum penicillinases Extended-spectrum oxacillinases Extended-spectrum β-lactamases
Imipenem	Metallo- β-lactamases Alterations of OprD porin (reduced outer membrane permeability)
Meropenem	Active efflux Metallo- β-lactamases Alterations of OprD porin (reduced outer membrane permeability)
Aminoglycosides	Active efflux Aminoglycoside-modifying enzymes Ribosomal methylation
Fluoroquinolones	Active efflux Mutations in topisomerases

The production of β-lactamases, efflux pumps and a low permeability of the outer membrane are all intrinsic to *P. aeruginosa*, whilst the modification of targets, expression of porins and expression of inactivating enzymes for aminoglycosides are all mechanisms that are acquired from other bacteria (Riou *et al.,* 2010). Efflux pumps are a widespread component of resistant strains and work alongside the low permeability of the outer membrane to form resistance to aminoglycosides, β-lactams, macrolides, fluoroquinolones and tetracycline (Schweizer, 2003; Poole *et al.*, 2001). Although found to confer low levels of resistance, Niga *et al.*, (2005) demonstrated that the overexpression of efflux pumps (induced in response to antimicrobial pressure) lowers the intra-bacterial antibiotic concentration and thus favours the emergence of target mutations in DNA gyrase (GyrA), conferring high level fluoroquinolone resistance. Such other target mutations as the methylation of 16S rRNA confers resistance to aminoglycosides and is thought to have been acquired from Gram-positive bacteria (Liou *et al.*, 2006). Recently, Su *et al.,* (2010) demonstrated that the development of fluoroquinolone resistance determinants, such as efflux pumps, is a multifaceted process and that pre-existing cellular pathways act in support of inducible genetic mutations, working together to produce resistance. This information may be applied to the development of novel strategies to prevent the emergence of fluoroquinolone resistance in other bacteria.

P. aeruginosa present in biofilms can be up to a thousand times more resistant, exhibiting an increased tolerance to antimicrobials as well as withstanding the effects of the body's immune system. Indeed chronic lung infections in cystic fibrosis patients are caused by biofilm-growing mucoid strains and the development of resistance to all available antibiotics has been reported (Hoiby *et al.*, 2010). Typical resistance mechanisms such as those previously mentioned do contribute to the overall survival of biofilms, however the increased mutation frequency, altered growth rate, quorum sensing and persister variants are considered to be integral to the increased resistance (Harmsen *et al.*, 2010). Although a rather obvious resistance mechanism would be the barrier provided by the exopolysaccharide matrix many reports have provided conflicting results; Vrany *et al.*, (1997) demonstrated that the penetration of ciprofloxacin and levofloxacin through a *P. aeruginosa* biofilm was not significantly delayed, whilst Suci *et al.,* (1994) indicated that the diffusion of ciprofloxacin was significantly delayed in the presence of a biofilm. With this in mind, Walters *et al.* (2003) concluded that the delayed penetration of antimicrobials was not a significant factor in the increased resistance of biofilms, but suggested that low growth rate and oxygen limitation were more accountable. Indeed studies by Folsom *et al.*, (2010) and King *et al.*, (2010) support this hypothesis. The elevated antimicrobial resistance of biofilms is widely considered to be multifactorial, with many studies emphasising the importance of altered phenotypes (persister cells in particular are key to the survival of populations) and horizontal gene transfer (allows acquisition of resistance genes and the development of multi-drug resistance in an entire population) (Molin *et al.,* 2003; De Groote *et al.*, 2009).

Multidrug resistant *Escherichia coli*

Escherichia coli is not traditionally associated with nosocomial infections, however due to the acquisition of resistance determinants, it has emerged as the leading Gram-negative pathogen responsible for bloodstream and urinary tract infections. In 2008 *E. coli* exhibited a Europe-wide increase of resistance to fluoroquinolones, third generation cephalosporins and aminoglycosides. Of more importance, an increased

frequency of multidrug resistance was observed, identifying it as a major threat to public health (EARRS, 2010).

CTX-M extended spectrum β-lactamase (ESBL) producing *E. coli* have rapidly emerged over the past decade and are largely associated with a nosocomial setting (Rodriguez-Bano *et al*., 2006). CTX-M enzymes render *E. coli* resistant to a variety of β-lactams, specifically cefotaxime, and are transferred via plasmids that can also include resistance genes to numerous unrelated classes of antibiotics (Canton *et al.*, 2006). Recent studies have demonstrated the acquisition of multiple antibiotic plasmid genes by CTX-M producing *E. coli*; Jiang *et al.*, (2008) and Cattoir *et al.*, (2008) reported the acquisition of quinolone resistance genes (qepA, qnr, and aac(6')-Ib-cr) whereas Baudry *et al.*, (2009) reported the presence of the aminoglycoside resistance gene aac(3')-II. Whilst aac(6')-Ib-cr and aac(3')-II both code for aceyltransferases, qepA codes for a quinolone efflux pump and qnr codes for Qnr peptides (pentapeptide repeat proteins) which protect DNA gyrase and topisomerase IV from inhibition; this highlights the variability of resistance mechanisms exploited by *E. coli* (Jiang *et al.,* 2008; Baudry *et al.*, 2009). The accumulation of the aforementioned resistance genes has given rise to multidrug resistant CTX-M producing *E. coli* thus limiting therapeutic options. Furthermore, the emergence of carbapenem (so-called drug of 'last resort') resistance among CTX-M producing *E. coli* is also a cause for serious concern. The newly identified New Delhi metallo- β-lactamase (NDM-1) confers resistance to all suitable antibiotics for infections caused by CTX-M producing *E. coli*, except colisitin and tigecycline (Kumarasamy *et al*., 2010). Moreover, there is increasing evidence of its ability to spread; isolates displaying this carbapenemase have been found in the UK, India, Pakistan and Australia (Kumarasamy *et al*., 2010; Poirel *et al*., 2010). These findings confirm the ability of resistance determinants to spread rapidly and be co-selected for in a hospital environment, adding further to the clinical issue of antibiotic resistance.

Conclusion

The issue of hospital-acquired infections caused by antibiotic resistant bacteria is one that should, by under no means, be underestimated. The ability of such notorious pathogens as *S. aureus and E. faecium* to evade the effects of newly synthesised, as well as traditional antimicrobials, is incredibly detrimental to public health. Furthermore, the development of multidrug resistant *C. difficile, P. aeruginosa* and *E. coli* leaves very limited treatment options. Research efforts to elucidate the mechanisms of resistance have provided key insights, however further expansion of this is required to gain a comprehensive understanding, thus allowing the development of new, alternative treatments and perhaps more accurately predict the evolution of resistance. Current outbreaks of nosocomial infections caused by resistant bacteria should drive efforts to prevent such emerging outbreaks as VRSA and linezolid-resistant enterococci from resulting in a global problem. The key, and first, control strategy to deploy would perhaps be the strict administration of antibiotics in a bid to reduce the current selection pressure found in hospitals and maintain antimicrobial susceptibility at an appropriate level.

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