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# For debate

# The problem with cephalosporins

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**The cephalosporin antibiotics have become a major part of the antibiotic formulary for hospitals in affluent countries. They are prescribed for a wide variety of infections every day. Their undoubted popularity relies upon lesser allergenic and toxicity risks as well as a broad spectrum of activity. It is the latter feature, however, that encourages the selection of microorganisms that are resistant to these agents. There are long-term implications for the treatment and control of this heterogeneous group of superinfections. When clinicians evaluate a septic patient, it is understandable that they choose empirical therapy with a cephalosporin whilst awaiting microbiology and other tests, since bacterial identification and antimicrobial testing still usually require 24–48 h. The broad-spectrum capability of these drugs, however, encourages rapid overgrowth of some microorganisms that are neither eliminated nor inhibited by therapy. These organisms not only have pathogenic potential, they may also be multiply resistant to antibiotics. This review discusses the evidence that cephalosporin usage is the most important factor in the selection and propagation of microorganisms such as *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, multiply resistant coliforms and vancomycin-resistant enterococci, the continuing increase of which threatens the future of antimicrobial therapy.**

## Introduction

Although widely accepted as broad-spectrum antibiotics, cephalosporins are not active against all the bacteria commonly isolated in a hospital microbiology laboratory.[1] Organisms that are not inhibited by cephalosporin therapy consequently overgrow, with varying potential to cause infection.[2,3] Some of these are instantly recognizable as pathogens; others, although originally regarded as commensal or of low risk status, have subsequently been shown to cause disease.[4] Furthermore, there is an association between cephalosporin usage and the emergence of multiply-resistant organisms.[2,5–9]

Microorganisms selected by cephalosporin therapy include commensal organisms such as coagulase-negative staphylococci (CNS), *Pseudomonas aeruginosa*, enterococci and *Candida albicans*, and organisms of more established pathogenicity, e.g. *Clostridium difficile*, penicillin-resistant pneumococci, multiply-resistant coliforms and methicillin-resistant *Staphylococcus aureus* (MRSA). Some of these organisms are constitutively resistant to cephalosporins while others have acquired resistance, usually as part of a multiple resistance package. This review discusses the evidence for a link between cephalosporins and overgrowth of certain microorganisms, including those that are multiply resistant to antibiotics.

## Inherently resistant microorganisms

### *Coagulase-negative staphylococci*

CNS are the most prevalent skin commensals. Hospitals are a source of CNS, which includes carriage by patients and staff,[10–12] and a reservoir in the hospital environment.[13] There appears to be a relationship between antibiotic usage and antibiotic resistances of CNS in hospitals.[14,15] Isolates from patients are generally multiply-resistant due to the continued heavy exposure of the hospital, staff and patients to antibiotics.[11,16] Patients newly admitted to hospital tend to acquire hospital CNS within hours of their admission, especially if prescribed antibiotic therapy.[11,17,18]

Experimental models show that the selective pressure exerted by broad-spectrum cephalosporins brings about a rapid overgrowth of staphylococci that are resistant to the antibiotics used.[19] This is also seen clinically, as most antibiotic-resistant hospital CNS are resistant to methicillin

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and are therefore relatively unaffected by cephalosporins.[20] They consequently proliferate upon and within patients receiving these antibiotics.[17,18,21] This property is not exclusive to the cephalosporins, as any antibiotic could theoretically have the same effect.[15,16] It is very difficult to rank antibiotics, let alone individual $\beta$-lactam agents, according to their selective ability, but there have been studies showing that methicillin resistance in CNS is significantly associated with therapeutic and prophylactic use of cephalosporins.[15,18,22]

Methicillin-resistant CNS encouraged by cephalosporin therapy may have clinical implications for some patients.[6,17,18,22] CNS are commonly associated with infections of artificial prostheses, including plastic catheters, and will generate persistent low-grade infections unless the prosthesis is removed.[23] Current management usually involves removal of such prostheses under glycopeptide cover, which increases the overall usage of these antibiotics.[24]

Many hospitals in developed countries consume large amounts of cephalosporin antibiotics, particularly in surgical departments as the preferred choice for prophylaxis.[25] This is being questioned at the present time for some specialities, because of concerns about the increasing prevalence of methicillin-resistant staphylococci. Some centres are already advocating a change from first- and second-generation cephalosporins to glycopeptides, but this move may be premature for others.[22,26,27] These antibiotics are both expensive and toxic, and their use has been discouraged following the emergence of glycopeptide-resistant enterococci (GRE) and more recently, glycopeptide intermediately resistant *S. aureus* (GISA).[28,29]

### *Oxidative non-fermentative Gram-negative bacilli*

*P. aeruginosa* is another common isolate from patients. Ceftazidime and some newer cephalosporins aside, most cephalosporins encourage overgrowth of this organism because it is inherently resistant to these agents.[2,30] Consumption of cephalosporin antibiotics in a hospital is associated with an increase in the isolation of *P. aeruginosa.*[31,32] Ceftazidime use itself leads to a significant reduction in susceptibility of *P. aeruginosa* to this antibiotic.[5,9,33] If its use is subsequently restricted, the proportion of susceptible *P. aeruginosa* increases once again.[5] Oral cephalosporins prescribed for urinary tract infections select for *P. aeruginosa*, which overgrows and may then mask the identity of the original pathogen.[34] Repeat swabs consequently incite inappropriate treatment for an organism that is not necessarily the primary pathogen and may only be of low clinical significance. Therapy with second- or third-generation cephalosporins also encourages overgrowth of *Stenotrophomonas maltophilia*.[31,35,36] This organism may require additional treatment, although therapy is difficult because it is often multiply resistant to antibiotics.[37]

Ceftriaxone or ceftazidime therapy selects resistant mutants from pre-existing susceptible strains of *Pseudomonas*.[31,33,38] In addition, these resistant mutants may be able to secondarily transfer the capacity for extended-spectrum $\beta$-lactamases (ESBLs) into Enterobacteriaciae.[38] There are few therapeutic options for infections caused by these strains, and the only effective oral agents (quinolones) are losing ground because of increasing resistance.[39]

### *Enterococci*

Enterococci are also associated with cephalosporin therapy.[3,19,40–45] These faecal-type streptococci first provoked interest as emerging pathogens in both hospital and community in the 1980s.[4] Most infection occurs in the urinary tract, but patients who have received, or are receiving, parenteral cephalosporins appear to be at risk from enterococcal infections in a variety of sites, including blood.[3,43,45] This is because enterococci are inherently resistant to cephalosporins and are able to colonize gastrointestinal sites previously populated by cephalosporin-susceptible organisms.[19,41,46] This is particularly well illustrated by current theories regarding colonization resistance.[47] Prescribing an antibiotic that decreases colonization resistance of the alimentary canal allows increased population densities (overgrowth) of potentially pathogenic bacteria. This correlates with mucosal invasion followed by translocation to lymph nodes. Overgrowth may also be associated with development or acquisition of resistance to the antibiotic prescribed.[47]

The majority of healthy volunteers given cephalosporins acquire a substantially increased proportion of enterococci in the gastrointestinal tract.[30,41,48] This is also seen in patients.[44,45,49] Animal studies describe profound effects of cephalosporin agents on colonization resistance, although antibiotics other than cephalosporins are also implicated.[50] It is difficult to apportion the effects of different antibiotics on colonization resistance because there are so few detailed studies specifically examining this. Some antibiotics, however, seem to be better able to maintain the status of the indigenous gastrointestinal flora than others.[41,51]

Patients tend to be more vulnerable than volunteers to changes in colonization resistance and enterococcal overgrowth precedes infection of the urinary tract, wounds, catheter sites and/or blood.[3,43,45] As with *Pseudomonas*, therapeutic options for clinically significant isolates are limited, and management has been further complicated by an increase in resistance to amoxicillin and gentamicin.[52] GRE are virtually untreatable,[53] and are discussed further below.

### *Clostridium difficile*

*C. difficile* has long been associated with a range of clinical diseases, from antibiotic-associated diarrhoea to pseudomembranous colitis. Overgrowth of *C. difficile*, with or

without clinical symptoms, is not exclusively associated with cephalosporins and has been reported following administration of many other antibiotics.[54] However, overgrowth occurs more commonly after cephalosporin therapy.[19,41,55–57] Healthy volunteers given oral cephalosporins virtually all excrete *C. difficile* after 10 days of therapy.[58] Treatment options for compromised patients usually include oral vancomycin or metronidazole, or both. Treatment failures occur frequently and precipitate the patient into and out of isolation, such is the propensity for spread of this spore-forming anaerobe.[59] The organism may also be associated with outbreaks, which are debilitating to patients and costly for the hospital.[57,60,61]

There has been a substantial increase of *C. difficile* in the UK over the last decade.[62] It is possible that the British Thoracic Society endorsement of cephalosporins as first-line treatment for community-acquired pneumonia has played some part in the increase of this organism.[63–65] A recent review states that the association between cephalosporins and *C. difficile* overgrowth is now so well established that cephalosporins should not be prescribed in care of the elderly units.[64] This view has already been successfully transcribed into clinical practice, whereby restriction of cephalosporin use has resulted in a decrease in the numbers of patients with *C. difficile.*[66,67]

### *Candida albicans*

Overgrowth is not solely a bacterial domain. *C. albicans* awaits any opportunity afforded by antibacterial therapy, yeast infections often following a course of antibiotics. Antibacterial therapy has consistently been shown to be a major independent risk factor for the development of systemic candidosis.[68,69] By no means exclusive to treatment with cephalosporins, *Candida* overgrows following exposure to most antibiotics.[19,30,41] It is those with the broadest spectrum of cover, however, that are more likely to encourage overgrowth.[44] Quinolones and aminoglycosides, for example, do not induce candidosis as rapidly or as often as cephalosporins.[70,71] When volunteers are given oral cephalosporins, most become colonized by yeasts within 2 or 3 days of starting therapy;[30] in another study, a group given amoxicillin did not show any increase in colonization with *Candida*.[19] Patients treated with cefaclor, cefalexin, cefradine, cefuroxime, ceftriaxone, latamoxef or ceftazidime have an increased risk of developing *Candida*.[2,44] Volunteers receiving parenteral ceftriaxone also show an overgrowth of yeasts in faecal flora.[48] Surgical patients treated with cephalosporins demonstrate increased colonization with *Candida*.[72] Patients are commonly found to have *Candida* from a throat swab soon after cefotaxime is initiated. Such patients are also at risk of candiduria, especially if the patient is catheterized.[73] Candidaemia may follow if colonization of superficial sites and/or the urine is ignored.[68,74] The more sites growing *Candida*, the higher the risk of invasive candidosis.[75] Treating clinically significant candidal overgrowth is difficult, especially when there are so few antifungal agents available.

There has been a huge increase in infections due to *Candida* over the past 20 years[76,77] and there is no sign that numbers are abating.[78] In hospital patients, the rate of blood-stream infection due to *Candida* spp. increased by almost 500% during the 1980s.[76,77] Whilst there may be several different reasons for this, including greater awareness, more clinical interventions and better laboratory techniques, the increasing amount of *Candida* mirrors the increasing use of cephalosporins introduced over this period.

## Recent trends in the aetiology of hospital-acquired infections

There has been a major shift in the aetiology of hospital-acquired infections during the 1980s in contrast to the 1970s, that is, an increase in the laboratory isolation of CNS, *Candida*, *S. aureus*, enterococci, *P. aeruginosa* and *Enterobacter* between 1980 and 1986–1989.[77,79] Taken as a whole, the shifts are away from more easily treated pathogens towards more resistant pathogens with fewer options for therapy.[79,80]

With the complexity and choice of antibiotic therapy nowadays, it is difficult to find specific evidence for the association of commensal overgrowth with the cephalosporin antibiotics alone. One study looked at hospital-acquired bacteraemia in an adult intensive care unit over 25 years (1971–1995).[32] Here, the use of amoxicillin plus gentamicin was gradually replaced by cephalosporins as first-line choice for the treatment of bacteraemic patients. During the last 5 years of the study, the number of bacteraemias increased two-fold, largely owing to increased isolation of enterococci, CNS, intrinsically antibiotic-resistant Gram-negative organisms (particularly *P. aeruginosa*) and *Candida*. Whilst the cephalosporins were introduced in the early 1980s, their prescribing frequency did not equal that of gentamicin until the 1990s. The organisms highlighted are the same as those already mentioned as being associated with cephalosporin therapy. The authors attributed the change in the spectrum of organisms to the changes in antibiotics used over the time period studied.[32]

An additional study shows that if cephalosporin usage is reduced as part of an overall reduction in antimicrobial prescribing, there is a decrease in hospital-acquired infections, namely, enterococcal and selected Gram-negative bacteraemias, and MRSA and *S. maltophilia* colonization or infection.[81] Others have documented the association of cephalosporins with staphylococci, enterococci, multiply-resistant Gram-negative bacilli, yeasts and *C. difficile*.[2,82,83]

The next section describes a group of multiply-resistant bacteria rapidly increasing in hospitals throughout the world. It appears that cephalosporin usage selects for and encourages propagation of these organisms. It is even

possible that the cephalosporin antibiotics play a role in the molecular initiation of resistance for some.

## Microorganisms with acquired resistance

### *Extended-spectrum β-lactamase-producing coliforms*

β-Lactamases are the major determinants of resistance to β-lactam antibiotics.[84] All Gram-negative bacteria elaborate chromosomally mediated β-lactamase enzymes. These are typically low level in coliforms isolated from human-free environments but may be induced in a wide variety of species by exposure to β-lactam drugs.[85,86]

The prevalence of β-lactamases has forced pharmaceutical companies to seek alternative agents that are resistant to β-lactamase attack.[87] Cefotaxime and ceftazidime were initially regarded as indestructible from plasmid-mediated β-lactamases, but this belief has since been shattered following a cascade of reports describing plasmid-mediated resistance to both of these drugs.[88,89] There are now increasing numbers of plasmid-mediated ESBLs described every year. Almost all of them are derivatives of the well-known TEM and SHV-1 β-lactamases.[90] Variants of these inactivate third-generation cephalosporins and monobactams, having arisen by spontaneous mutation and being only marginally different in amino acid sequence from the parent enzymes.[1] An additional mechanism of resistance is the capture on plasmids of normally chromosomal genes from *Enterobacter cloacae*, *Citrobacter freundii* or *P. aeruginosa*, which can provide *Klebsiella pneumoniae* or *Escherichia coli* with resistance to α-methoxy-β-lactams (cefoxitin and cefotetan) as well as to oxyimino-β-lactams (cefotaxime, ceftriaxone and ceftazidime).[91] A resistant organism isolated during therapy to one cephalosporin may thus demonstrate reduced susceptibility to other antibiotics, not necessarily within the same chemical class.[92]

Multiply-resistant coliforms are associated with high-level usage of cephalosporins, particularly cefotaxime, ceftriaxone and ceftazidime.[5–9,44,87,93–97] These antibiotics induce and select for ESBL coliforms (ESBLC).[87,95,98] If cephalosporins are avoided, there is less chance of selecting these highly resistant bacteria, and coliform susceptibility rates rise.[5,99] At a hospital in New York, multiply-resistant *E. cloacae* isolates from the intensive care unit increased dramatically between 1988 and 1990.[97] As a response, use of ceftazidime was severely restricted in favour of piperacillin in combination with an aminoglycoside. Following this change, susceptibility of *E. cloacae* isolates to ceftazidime increased from 54% to 75%, whilst the total number of multiply-resistant *E. cloacae* fell. No other major changes in susceptibility patterns were seen.[97]

Some resistant coliforms merely colonize patients; others invade to cause infection.[8] Yet other epidemic strains spread to cause outbreaks of virtually untreatable disease.[100,101] The location of antibiotic resistance mechanisms on plasmids facilitates easy spread between species and genera, and is most likely to occur in the gastrointestinal tract.[46,102]

Some physicians have already recognized the fact that cephalosporin usage may play a role in the selection of ESBL and therefore prescribe amoxicillin in conjunction with an antibiotic of another class for first-line treatment of community-acquired pneumonias.[87] Similarly, some surgeons have reverted to the 'old-fashioned' combination of amoxicillin and gentamicin for surgical patients.[87] The latter combination is less likely to encourage overgrowth of susceptible enterococci, yeasts and *C. difficile*, than would occur if a cephalosporin was the antibiotic of choice.[3,19,57,72]

Hospitals contain a concentrated reservoir of resistant coliforms, but a dilute version exists in the community. The path between the hospital and the community runs both ways.[103] Even patients with no prior hospital contact can display clinically significant infection with multiply-resistant coliforms.[104] Critically ill patients in intensive care units rapidly acquire such organisms, even if not previously colonized, and a battle often ensues between microbiologist and organism for time to allow the patient to recover from initial pathology before succumbing to hospital-acquired resistant microbes.

### *Penicillin-resistant pneumococci*

Clinically significant infection with penicillin-resistant pneumococci (PRP) is prevalent worldwide.[105] At present, the median MIC for strains in the USA lies between 0.05 and 0.1 mg/L and is continuing to rise. Most infections with intermediately resistant pneumococci (MIC 0.1–1.0 mg/L penicillin) are treatable with increased doses of penicillin, but isolates are now showing high-level resistance (>2.0 mg/L).[106] Some of these demonstrate resistance to third-generation cephalosporins, and this proportion is also increasing.[106]

PRP are associated with extensive prior antimicrobial therapy,[107] particularly with β-lactam antibiotics.[108–112] They may be selected at the infection site or in the nasopharynx.[109] Resistant strains may even reside in the nasopharynx before treatment is initiated and are then encouraged by the antibiotic given. It is also possible that penicillin-susceptible strains are transformed *in vivo* to strains that have a lower susceptibility to penicillin.[113] Any antibiotic in theory has the potential to select resistant strains provided that the local concentration of the drug is high enough to inhibit susceptible cells whilst encouraging a resistant subset.[109] The risk of selection is increased if patients are under-dosed or given prolonged courses of antibiotics, or if drugs with inadequate activity against pneumococci are prescribed.[114,115]

The aminopenicillins have been widely regarded as being disproportionately responsible for selecting PRP, but this may be only because the emergence of this pathogen

coincided with increased consumption of these antibiotics.[116] It is entirely possible that increased prescribing of other antibiotics, e.g. cephalosporins, macrolides and co-trimoxazole, is equally or more important than aminopenicillins in the promotion of PRP.[116–119] Studies examining the effect of antibiotics on the carriage of PRP are often flawed in their design and conclusions.[109] Authors do not often calculate the relative selective pressures of different classes of antibiotics;[117–119] in many studies, investigators fail even to identify the specific antibiotics given, referring only to the generic term 'antibiotics'.[109] Even more unusually, some authors identify the classes of antibiotics that select resistant strains, but fail to complete the analysis. In one of these studies, co-amoxiclav was shown to be associated with a minimal increase in the incidence of PRP carriage (from 14% to 16%) whilst the use of other unidentified antibiotics was associated with a much greater increase in PRP incidence (from 39% to 70%).[120]

It is indisputable that aminopenicillins promote the carriage of PRP, because in common with other $\beta$-lactams, they select PRP strains already present in the oropharynx. It is also the case that there are other penicillin-resistant streptococci present in the oral cavity, which could serve as reservoirs for resistance genes that lead to alterations in pneumococcal penicillin-binding proteins (PBPs).[121]

There are some studies that detail a connection between cephalosporins and PRP.[2,105,109,122–124] One compares the efficacy of cefixime versus co-amoxiclav in children with acute otitis media.[124] Cefixime was associated with a rate of selection of PRP of 41.9%, compared with 15.9% for co-amoxiclav.[124] The selection of PRP by oral cephalosporins, both *in vitro* and *in vivo*, can be explained by their reduced activities against PRP, as well as by the fact that the modification of a single penicillin-binding protein, PBP 2x, may result in a marked increase in MICs.[124–126] Selection by cephalosporins occurs at higher frequencies than that by amoxicillin.[125] Even very potent broad-spectrum cephalosporins are capable of selecting PRP.[117] In a study whereby a single dose of ceftriaxone was compared with a 10 day course of co-amoxiclav for children with acute otitis media in an area of high PRP prevalence, nearly twice as many PRP were selected by the cephalosporin as by co-amoxiclav (27.4% and 14.5%, respectively).[117]

Other work indicates that oral cephalosporins promote mutants with higher MICs of parenteral third-generation cephalosporins than of penicillin.[105] Oral cephalosporins are often prescribed for streptococcal pharyngitis, where colonizing pneumococci normally reside. Use of these agents may be responsible for the sudden increase in PRP recorded by a community survey in Northern Ireland;[127] consumption has certainly been correlated with the prevalence of high-level penicillin resistance in Spain.[128] The inference is, therefore, that cephalosporins select for and encourage resistance to $\beta$-lactam antibiotics in pneumococci colonizing the oropharynx.[109] Furthermore, PRP have greater potential to spread than susceptible strains.[129] Concurrent resistance to other antibiotics, including macrolides and co-trimoxazole in multiply-resistant strains, will not only select still greater numbers of resistant populations in the nasopharynx, but may also lead to clinical failures, thereby increasing the risk of dissemination of PRP.[130] The pathogenicity of this organism is such that the increasing incidence worldwide is of major concern to clinicians.

## *MRSA*

The prevalence of MRSA is also giving cause for concern.[131] *S. aureus* itself has always been regarded as a pathogen and now there are only a few remaining antibiotics effective against resistant strains. Potential usage of any member of the $\beta$-lactam group is excluded once *S. aureus* becomes resistant to methicillin.[1] There is a steady increase of new cases nationally and an increasing proportion of MRSA from total numbers of staphylococcal bacteraemias.[132]

MRSA was first described in 1961 in Britain, but despite fear of spread, there were only sporadic outbreaks in the 1960s and 1970s.[133–135] Cephalosporins were widely introduced in 1980 and the first epidemics of MRSA were reported in London during the middle 1980s.[136] By 1990, most parts of the UK were affected.

The Japanese experience cites the introduction of second- and third-generation cephalosporins in the early 1980s as playing a significant part in the emergence and spread of MRSA in Tokyo hospitals.[137–139] The steady increase of MRSA in Europe, including Britain and Italy, has also been attributed to the use of cephalosporins.[40,122,135,140–142] A report from America describes a community outbreak of MRSA among iv drug abusers who self-administered cephalosporins for prophylactic purposes.[143]

At least three mechanisms account for methicillin resistance in *S. aureus*: production of PBP 2a or 2′ encoded by the chromosomal *mec*(A) gene, production of modified PBPs and inactivation of methicillin by $\beta$-lactamase.[144] There is insufficient evidence to prove the molecular role played by antibiotics in the acquisition of these mechanisms but it is widely believed that antibiotics are associated with the induction, selection and propagation of MRSA.[137,138,140,145–151] The induction hypothesis originates firstly from training procedures, whereby methicillin-susceptible *S. aureus* (MSSA) is cultured in broth containing sub-MIC levels of $\beta$-lactam antibiotics, in particular, cefazolin and ceftizoxime.[138] It is possible to create MRSA (MIC $>$ 1000 mg/L) from such experiments, although rather more difficult to transpose them into a clinical context. Methicillin-resistant clones can also be identified from *S. aureus* specifically resistant to cephamycin antibiotics,[146] and serial exposure of *S. aureus* to cefalexin discs induces the development of staphylococci cross-resistant to cefalexin and methicillin.[152] The latter work also showed that once induced, the capacity for methicillin resistance was not easily lost.[152] Considering selection, some authors

specifically cite cephalosporin therapy as a major factor in the appearance of MRSA.[149–151] Once established, continued use of cephalosporins encourages the spread of the organism.[137,151]

It is reasonable to assume that all members of the $\beta$-lactam class of antibiotics have some ability to induce methicillin resistance in staphylococci, albeit difficult to prove.[152] It is even more difficult to apportion the ability for the selection process between individual $\beta$-lactam agents.[15,153] If the capacity for generating MRSA is regarded as similar between $\beta$-lactam agents, however, then cephalosporins shoulder much of the responsibility owing to the frequency of their use.

There are further issues that may be important in the evolution of MRSA. We may accept that the most plausible hypothesis is repeated exposure of *S. aureus* to $\beta$-lactam antibiotics,[152,154] but it is also possible that inadequate doses, or too short a course, of the same agents may fail to eradicate infection with MSSA whilst presenting the $\beta$-lactam ring to the organism as a template or trigger. Poor prescribing, or non-compliance on the part of the patient, would encourage bacterial mutation to produce effective resistance mechanisms or, more likely, facilitate the clonal expansion of a member of the population already with the genetic capabilities for methicillin resistance.[154] But these are probably not the only factors by which MSSA becomes MRSA. It is also possible that the methicillin resistance genes are transferred to *S. aureus* from *Staphylococcus epidermidis.*[138,155,156]

Antibiotic resistance characteristics can be transferred between coagulase-negative and coagulase-positive staphylococci and this includes methicillin resistance.[156] Most hospitals have endemic methicillin-resistant *S. epidermidis* (MRSE) in the environment as well as colonizing staff and patients,[10,12,13,157] and newly admitted patients soon become colonized with multiply-resistant CNS.[11,12,16,17] Patients may even be admitted with MRSE on the skin at the proposed operation site, which, the authors suggested, should question the use of prophylactic cephalosporins.[22] This particular study concerned patients admitted for prosthetic hip implants, in whom it was shown that the most common post-operative infections were caused by methicillin-resistant coagulase-negative staphylococci.[22]

The consequence of cephalosporin prophylaxis is illustrated by two clinical studies of surgical patients, the first of which showed that MRSE was detected in high numbers on the skin of surgical patients within 5 days of exposure to per-operatively administered cephalosporins.[18] The second showed that just three doses of cefuroxime encouraged the appearance of MRSE from aortic graft recipients within 1 week.[158] Once colonized, further usage of $\beta$-lactam antibiotics exerts the selection pressure required for continued increase in methicillin-resistant staphylococci.[14,15,137,147,151] As it is the case that all persons are colonized with *S. epidermidis* and one in three persons are colonized with *S. aureus*, the propensity for selection and spread of methicillin-resistant staphylococci and the potential for genetic exchange between staphylococcal species becomes immediately apparent.[137,155,156]

There is a direct association between MRSA and cephalosporins. Asensio *et al.*[159] showed that patients who had received treatment for >5 days with cephalosporins were three times more likely to acquire MRSA than those who had not received these agents. This agrees with other studies, which detail a significant association of cephalosporins with the acquisition of MRSA.[149–151,160] Conversely, a study demonstrating the effects of reducing cephalosporin usage in three acute medical wards for the elderly showed that the number of MRSA infections was reduced by half; there was also a 42% drop in the number of *C. difficile* infections.[66] Another reported a decline in the number of MRSA isolates from 35% to 23%, following the introduction of control strategies including a decision to decrease the use of cephalosporins in favour of piperacillin–tazobactam.[161] MRSA appears to be a sensitive indicator of the quality of hospital hygiene overall,[162] as it is associated with other hospital organisms of concern.[66,81,161] If a hospital manages to control MRSA, it controls other hospital organisms as well.[162]

Recently, there has been a report published detailing the deaths of four children from MRSA infections, three of whom had originally received a cephalosporin.[163] Cephalosporins are only poorly active against MRSA;[164–166] it is possible that usage of these antibiotics not only selects for MRSA, but encourages enhanced virulence.[151,161] Certainly, prophylactic cefazolin is a risk factor for deep surgical wound infections with borderline oxacillin-susceptible strains of *S. aureus*.[167]

## *GRE*

Enterococci have also become a significant cause of hospital-acquired infection over the past 20 years.[4] Natural resistance to penicillin and cephalosporins has been further complicated by additional resistance to amoxicillin, co-amoxiclav, gentamicin and now glycopeptides.[53,168] There are virtually no antibiotics available for the treatment of clinically significant resistant enterococci and no means whereby asymptomatic carriers can be cleared.[28,169]

The association between cephalosporin therapy and enterococcal overgrowth has already been discussed. It follows, therefore, that vancomycin-resistant enterococci (VRE) are also associated with cephalosporin usage.[170,171] A recent report describes how hyperendemic VRE in a haematology unit was effectively eradicated by changing from ceftazidime to piperacillin–tazobactam as first-line treatment for febrile neutropenics. The reintroduction of ceftazidime was accompanied by a return of VRE, despite continued attention to hygiene and surveillance.[170] A study previously mentioned witnessed a drop in the number of VRE isolates from 16% to 5% after restricting cephalosporins in favour of piperacillin–tazobactam.[161] Outbreaks

of VRE may be controlled by switching from cephalosporins to aztreonam,[172] or from cephalosporins to aminoglycosides.[173]

VRE are also linked with the use of glycopeptides in hospitals[151,168,174] and in animals,[174,175] and this has been assumed to be the most important factor in the selection of these organisms.[171] However, if the indications for vancomycin therapy are examined, it is apparent that some of these indications are actually generated by the use of cephalosporins,[3,6,151,176] i.e. there is not only a direct association of cephalosporins with VRE, but an indirect one, whereby cephalosporins select other organisms that require treatment with vancomycin, which *then* leads to the selection of VRE.[151] Methicillin-resistant staphylococci, for example, already linked to the widespread use of $\beta$-lactam antibiotics, including cephalosporins, warrant treatment with iv vancomycin for seriously infected patients.[23,168,177] Even more vancomycin is used, for prophylactic purposes, if patients at risk are admitted to a hospital with endemic methicillin-resistant staphylococci.[22,26] Glycopeptide-susceptible enterococci, selected by cephalosporin use, occasionally require treatment with vancomycin, especially if resistant to aminoglycosides.[3,52] Patients with *C. difficile* colitis, another sequelae of cephalosporin therapy, also require treatment with vancomycin.[171,178] Thus, cephalosporin use is associated with several different organisms, the management of which may include vancomycin. Increased vancomycin usage could then select for vancomycin-resistant organisms including VRE. The *C. difficile* scenario can be considered further regarding the evolution of VRE, because a patient treated with cephalosporin antibiotics suffers concurrent overgrowth of both *C. difficile* and enterococci.[176,179] If severe diarrhoea ensues, therapy is usually with oral vancomycin. Thereby an abundance of naturally resistant enterococci are exposed to non-absorbable vancomycin and the gut provides an excellent site for selection.[46] (Vancomycin-resistant pediococci, leuconostoc and lactobacilli inhabit the mouth, gut and genital tract, but these do not appear to be the source of the genes encoding acquired resistance in enterococci.[180]) When faeces are screened for VRE, those patients found to be positive are often colonized with *C. difficile* as well; some even have MRSA to complete the package.[179] In summary, therefore, it may be that heavy usage of cephalosporins is the main driving force behind the increasing prevalence of VRE, rather than glycopeptides.

This hypothesis is also supported by studies that report that parenteral vancomycin does not appear to be an important risk factor for acquisition of VRE,[181,182] nor did the administration of copious amounts of oral vancomycin for an outbreak of *C. difficile* appear to generate a problem with VRE.[173] Furthermore, successful restriction of vancomycin prescribing has had no effect upon the occurrence of VRE in some centres.[183,184]

An added complication regarding VRE in a hospital concerns its ability to survive long-term in the environment.[185] It is worth promoting environmental hygiene in a hospital, especially since there are no effective protocols for clearing VRE from the human gastrointestinal tract.[28,186] The hospital environment may not be so temperate as the human gut, but may still provide an appropriate medium whereby organisms are able to transfer resistance genes.[187,188] Exchange of genes between Gram-positive organisms is well documented.[189,190] MRSA is another organism noted for its ability to survive in the hospital environment, but there is no evidence that resistant enterococci have contributed towards the appearance of glycopeptide-tolerant strains of MRSA.[191]

## *In vitro* studies on the pharmacodynamic effects of cephalosporins

So far, this review has focused upon the problem of selection and overgrowth of organisms associated with cephalosporin therapy. A common theme underlying this problem is the inability of these antibiotics to eradicate effectively key organisms, thus encouraging survival either with or without enhanced resistance to antibiotics. In this context, recent work on the mechanism of action of cephalosporin antibiotics offers some *in vitro* explanations for these clinical observations.[192]

Microscopic studies on the effect of $\beta$-lactam antibiotics on the bacterial cell at concentrations in excess of the MIC are able to distinguish between amoxicillin and cefalexin.[193] The sequence of events is inhibition of cell division followed by lysis; this occurs very quickly with amoxicillin, as cell growth barely reaches two cell units in length before the onset of lysis. With cefalexin, however, there is a significant period of filamentous growth before the cell lyses, usually as a result of a sudden rupture of the cell wall. Filaments can be observed in clinical specimens from patients with Gram-negative infections treated with a variety of $\beta$-lactams, and, in particular, the aminothiazolyl cephalosporins, e.g. cefuroxime, cefotaxime, ceftazidime and ceftriaxone. This property has prompted one author to state that bacterial growth is not actually inhibited by the filament-forming antibiotics and it follows that overall cell wall synthesis is not inhibited to any significant extent either.[194] It could also be said that the time utilized for filament-forming by the cephalosporins is time available for antimicrobial resistance induction, whereas the use of other agents, which induce early cell death, would reduce the risk of such an event.

Filamentous cells that develop in the presence of cephalosporins do eventually lyse as a result of a sudden rupture of the cell wall, usually at a point where cell division would normally have taken place.[193] Round cell formation and rapid lysis can be achieved by using higher concentrations of predominantly filament-forming anti-

biotics.[192,195] These antibiotics therefore do not precipitate the rapid cell lysis and bactericidal effects more usually associated with $\beta$-lactam agents, but promote the generation of aberrant forms that are able to survive *in vivo*. This may have clinical implications; first, as some bacteria may persist in patients following a course of one of these antibiotics; secondly, as survival may include the generation of tolerant or even resistant progeny, and thirdly, when assessing endotoxin release following exposure to antibiotics.[196]

Filament formation leads to a rapid increase in endotoxin production and, ultimately, endotoxin release when these cells lyse.[197] There are several studies to support this.[198–200] Higher doses of filament-forming antibiotics may alternatively produce the more fragile spheroplasts, which would tend to lyse and thus reduce the propensity for endotoxic shock.[196,201] It is possible, therefore, that the choice and dose of an antimicrobial agent for a patient with septic shock could be clinically crucial.[196]

## Conclusion

This review has attempted to piece together some of the suspicions surrounding a very widely used class of antibiotics. There is strong suggestive evidence that cephalosporins have played a major role in encouraging the organisms discussed. Countries, and even individual hospitals, that have enforced low usage of cephalosporins through education, strict antibiotic policies and prescribing penalties, are currently experiencing relatively low rates of multiply-resistant organisms.[66,135,141,142,161,202] Countries where cephalosporins are used more often have much higher rates of resistance.[122,137,141,203] It is, however, difficult to determine whether it is specific restriction of cephalosporin antibiotics in isolation that is responsible for the lower rates of resistance, or, indeed, an overall effect from controlling *all* antibiotic classes.[135] In defence of the former view, most of the references cited specifically target cephalosporins as key players in the link between antibiotic usage and prevalence of multiply-resistant organisms.

Compounding the problem is the fact that microbiologists have yet to define fully the mechanisms linking antibiotic usage and antibiotic resistance. Prescribing colleagues will almost certainly question how just one group of antibiotics alone, within the extensive $\beta$-lactam class, could be the most important driving force behind the continuing increase in resistant organisms, even allowing for broad-spectrum activity and popularity.[83,204,205] Convincing clinicians that antibiotic therapy should be more closely tailored to the patient requires scientific proof and this is not yet evident. Changing current antibiotic prescribing practices demands a strong microbiology presence, robust antibiotic policies, education and laboratory support, as well as a more careful evaluation of the infected patient and potential pathogen, confirmed or otherwise.[206]

If there is difficulty in convincing colleagues of the clinical and long-term benefits of decreasing cephalosporin use, then the funders of healthcare may offer a view. Aside from the cost benefits of reducing the amount of these drugs purchased, there are substantial savings to be made from lower numbers of patients who require treatment for the consequences of overgrowth resulting from cephalosporin therapy.[81,207]

It has been suggested that the clinical freedom enjoyed by the medical profession to prescribe what they like, when they like, should be reviewed for the prescription of antimicrobials.[208] Even experienced practitioners may not realize that giving a patient antibiotics affects not just that patient, but also their environment, and all the other people that come into contact with that environment.[204,209,210] Removing the right to prescribe antimicrobials freely would divide the medical profession and place a monumental burden upon microbiology and infectious disease specialists. There may also be repercussions for dentists and nurse prescribers. Even if this policy was implemented, inadequate infection control and antibiotic practices elsewhere in the world would erode any progress made in halting the increase of multiply-resistant organisms. The World Health Organization (WHO) must take some responsibility in promoting international discussion on antimicrobial use throughout the world.

A multifaceted approach to the control of multiply-resistant organisms is required.[202] There are a variety of strategies, some of which have already been mentioned, i.e. antibiotic policies, judicious use of antimicrobials by clinicians, laboratory support, WHO involvement and a strengthening of the role of microbiologists and infectious diseases physicians. Education for everyone is vitally important and in particular, perhaps, for medical students, for whom microbiology teaching should be re-prioritized within the undergraduate curriculum.[211]

Pharmacists play an important role in restricting over-the-counter sales and in the advice they offer. In the hospitals, they audit prescribing, evaluate adherence to policies and formulary and issue advice on iv-to-oral and antibiotic-stop mechanisms. The microbiology laboratory cannot provide instant identification of all microorganisms, but there are an increasing number of rapid diagnostic techniques available and 24 h processing of non-urgent specimens from high-risk patients can be introduced in order to avoid a sample languishing until the next working day. Laboratory computers should provide the data required for multiple audit and surveillance strategies for infection control. Pharmaceutical research, better definitions of infection and the dangers of prolonged prescribing are other potential control issues.[202]

In defence of the cephalosporin antibiotics, they provide useful activity against a number of common pathogens, and their low toxicity reassures clinicians and obviates the need for serum levels.[212] Patients allergic to penicillin sometimes rely upon a cephalosporin as the only agent available to

them. They should not be used for routine prophylaxis, however, but have their efficacy preserved with more rational prescribing.[22,213,214] There may well be an argument for revising the recommended adult doses of, in particular, the aminothiazolyl cephalosporins. Reduced bactericidal activity leading to the selection phenomena described may relate to inadequate dosing rather than an inherent therapeutic deficit. Tissue penetration and concentration at the site of infection are other factors to consider.

It is also pertinent to say that in the absence of cephalosporins, greater use of other antibiotics would have been required. Not only would these have very likely generated their own particular selection strategies, but also some agents would have almost certainly produced considerable toxic effects, far more so than the cephalosporins. It is unlikely, however, that we would have seen the prolific rise of multiply-resistant organisms if the cephalosporins had never been introduced. This is because few of the existing agents offered such broad-spectrum activity, with such low toxicity, and consequently would not have been universally prescribed. A greater range of antibiotics would have been utilized, diffusing the selection potential. It is the popularity of the cephalosporins, perhaps, that has become their downfall.

Resistance inevitably follows the introduction of a new antimicrobial. In intensive care units, the original promise offered by cephalosporins as broad-spectrum therapy was almost immediately eroded by the appearance of resistant organisms, and their use was supplanted by the use of quinolones. The legacy of heavy usage of these drugs, however, resulted in the appearance of multiply-resistant *Acinetobacter*. Now *S. maltophilia* flourishes, following the introduction of the carbapenems.[215] This ominous progression, played out over the past 20 years, can be likened to a worldwide chess game; as one piece is captured, another moves to threaten.[216] If we therefore shift prescribing choice away from the cephalosporins to another antibiotic class, bacteria will evolve resistance mechanisms to the new group chosen.[46] Resistance is the price one pays for having an antibiotic and using it, because nature abhors a vacuum and will fill it up if it can.[134] Ultimately, the future therapy of infection may almost certainly depend upon the immunologists, with construction of vaccines against virulence determinants or continued work on the development of cytokine inhibitors.[210] The latter are already showing positive benefits for patients in ITU.[217,218]

In conclusion, the selection pressure created by heavy usage of cephalosporin antibiotics over the last 20 years has generated a plethora of multiply-resistant organisms. The possible links between *C. difficile*, VRE and MRSA serve as a warning for potentially untreatable infection—with ESBLC providing the Gram-negative equivalent. The risks posed by overuse of cephalosporins remain only speculative, unless specific proof is forthcoming. By then, though, we may be contemplating the post-antibiotic era.

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## References

**1.** Livermore, D. M. (1991). Mechanisms of resistance to $\beta$-lactam antibiotics. *Scandinavian Journal of Infectious Diseases Supplement* **78**, 7–16.

**2.** Wise, R. (1997). $\beta$-Lactams: cephalosporins. In *Antibiotics and Chemotherapy*, 7th edn, (O'Grady, F., Lambert, P. H., Finch, R. G., Greenwood, D., Eds), pp. 202–55. Churchill Livingstone, New York.

**3.** Pallares, R., Pujol, M., Pena, C., Ariza, J., Martin, F. & Gudiol, F. (1993). Cephalosporins as risk factors for nosocomial *Enterococcus faecalis* bacteraemia. A matched case–control study. *Archives of Internal Medicine* **153**, 1581–6.

**4.** Moellering, R. C. (1992). Emergence of *Enterococcus* as a significant pathogen. *Clinical Infectious Diseases* **14**, 1173–6.

**5.** Bamberger, D. M. & Dahl, S. L. (1992). Impact of voluntary vs enforced compliance of third-generation cephalosporin use in a teaching hospital. *Archives of Internal Medicine* **152**, 554–7.

**6.** Pallares, R., Dick, R., Wenzel, R., Adams, J. R. & Nettleman, M. D. (1993). Trends in antimicrobial utilization at a tertiary teaching hospital during a 15 year period (1978–1992). *Infection Control and Hospital Epidemiology* **14**, 376–82.

**7.** Nicolle, L. E. (1988) Prior antimicrobial therapy and resistance of *Enterobacter*, *Citrobacter* and *Serratia* to third generation cephalosporins. *Journal of Hospital Infection* **11**, 321–7.

**8.** Follath, F., Costa, E., Thommen, A., Frei, R., Burdeska, A. & Meyer, J. (1987). Clinical consequences of development of resistance to third generation cephalosporins. *European Journal of Clinical Microbiology* **6**, 446–50.

**9.** Salacata, A. & Chow, J. W. (1993). Cephalosporin therapeutics for intensive care infections. *New Horizon* **1**, 181–6.

**10.** Maki, D. G. (1984). Comparison of organisms carried on the hands of hospital (ICV) personnel and non-medical control personnel. In *Program and Abstracts of the Twenty-fourth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 1984*. Abstract 521. American Society for Microbiology, Washington, DC.

**11.** Powell, M. & Sanderson, P. J. (1987). Resistant coagulase-negative *Staphylococci* in hospital patients. *Journal of Hospital Infection* **9**, 48–53.

**12.** Thurn, J. R., Crossley, K. B., Gerdst, A. & Baken, L. (1992). Dynamics of coagulase-negative staphylococcal colonisation in patients and employers in a surgical intensive care unit. *Journal of Hospital Infection* **20**, 247–55.

**13.** Maki, D. G., Alvarado, C. J., Hassemer, C. A. & Zilz, M. A. (1982). Relation of the inanimate environment to endemic nosocomial infections. *New England Journal of Medicine* **307**, 1562–66.

**14.** Lyytikainen, O., Vaara, M., Jarviluoma, E., Rosenqvist, K., Tiittanen, L. & Valtonen, V. (1996). Increased resistance among *Staphylococcus epidermidis* isolates in a large teaching hospital over a 12-year period. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 133–8.

**15.** Mouton, R. P., Hermans, J., Simoons-Smith, A. M., Hoogcamp-Korstanje, J. E. & Van Kungeren, B. (1990). Correlations between consumption of antibiotics and methicillin-resistance in coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy* **26**, 573–83.

**16.** Terpstra, S., Noordhoek, G. T., Voesten, H. G. J., Hendriks, B. & Degener, J. E. (1999). Rapid emergence of resistant coagulase-negative staphylococci on the skin after antibiotic prophylaxis. *Journal of Hospital Infection* **43**, 195–202.

**17.** Archer, G. L. & Armstrong, B. C. (1983). Alteration of staphylococcal flora in cardiac surgery patients receiving antibiotic prophylaxis. *Journal of Infectious Diseases* **147**, 642–9.

**18.** Kernodle, D. S., Barg, N. L. & Kaiser, A. B. (1988). Low-level colonization of hospitalised patients with methicillin-resistant coagulase-negative staphylococci and emergence of the organisms during surgical antimicrobial prophylaxis. *Antimicrobial Agents and Chemotherapy* **32**, 202–8.

**19.** Edlund, C. & Nord, C. E. (1991). A model of bacterial–antimicrobial interactions: the case of oropharyngeal and gastrointestinal microflora. *Journal of Chemotherapy* **3**, 196–200.

**20.** Chin, N. X., Neu, N. M. & Neu, H. C. (1990). Activity of cephalosporins against coagulase-negative staphylococci. *Diagnostic Microbiology and Infectious Disease* **13**, 67–9.

**21.** Monsen, T., Ronnmark, M., Olofsson, C. & Wistrom, J. (1999). Antibiotic susceptibility of staphylococci isolated in blood cultures in relation to consumption in hospital wards. *Scandinavian Journal of Infectious Diseases* **31**, 399–404.

**22.** James, P. J., Butcher, I. A., Gardner, E. R. & Hamblen, D. L. (1994). Methicillin-resistant *Staphylococcus epidermidis* in infection of hip arthroplasties. *Journal of Bone and Joint Surgery* **76**, 725–7.

**23.** Hamory, B. H., Parisi, J. T. & Hutton, J. P. (1987). *Staphylococcus epidermidis*: a significant nosocomial pathogen. *Journal of Infection Control* **15**, 59–74.

**24.** Raad, I., Davis, S., Khan, A., Tarrand, J., Elting, L. & Bodey, G. P. (1992). Impact of central venous catheter removal on the recurrence of catheter-related coagulase-negative staphylococcal bacteremia. *Infection Control and Hospital Epidemiology* **13**, 215–21.

**25.** Gorbach, S. L. (1989). The role of cephalosporins in surgical prophylaxis. *Journal of Antimicrobial Chemotherapy* **23**, *Suppl. D*, 61–70.

**26.** Mini, E., Nobili, S. & Periti, P. (1997). Methicillin-resistant staphylococci in clean surgery. Is there a role for prophylaxis? *Drugs* **54**, *Suppl. 6*, 39–52.

**27.** Sanderson, P. J. (1998). Prophylaxis in orthopaedic implant surgery—should we use a glycopeptide? *Journal of Antimicrobial Chemotherapy* **41**, 322–5.

**28.** Hospital Infection Control Practices Advisory Committee. (1995). Recommendations for preventing the spread of vancomycin resistance. *Infection Control and Hospital Epidemiology* **16**, 105–13.

**29.** Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T. & Tenover, F. C. (1997). MRSA clinical strain with reduced vancomycin susceptibility. *Journal of Antimicrobial Chemotherapy* **40**, 135–46.

**30.** Leigh, D. A., Walsh, B., Leung, A., Tait, S., Peatey, K. & Hancock, P. (1990). The effect of cefuroxime axetil on the faecal flora of healthy volunteers. *Journal of Antimicrobial Chemotherapy* **26**, 261–8.

**31.** Paull, A. & Morgan, J. R. (1986). Emergence of ceftriaxone-resistant strains of *Pseudomonas aeruginosa* in cystic fibrosis patients. *Journal of Antimicrobial Chemotherapy* **18**, 635–9.

**32.** Edgeworth, J. D., Treacher, D. F. & Eykyn, S. J. (1999). A 25-year study of nosocomial bacteremia in an adult intensive care unit. *Critical Care Medicine* **27**, 1421–8.

**33.** Jones, R. N. (1992). The current and future impact of antimicrobial resistance among nosocomial bacterial pathogens. *Diagnostic Microbiology and Infectious Diseases* **15**, *Suppl. 2*, 3S–10S.

**34.** Warren, J. W., Anthony, W. C., Hoopes, J. M. & Muncie, H. L. (1982). Cephalexin for susceptible bacteriuria in afebrile, long-term catheterized patients. *Journal of the American Medical Association* **248**, 454–8.

**35.** Khardori, N., Elting, L., Wong, E., Schable, B. & Bodey, G. P. (1990). Nosocomial infections due to *Xanthomonas maltophilia* (*Pseudomonas maltophilia*) in patients. *Reviews of Infectious Diseases* **12**, 997–1003.

**36.** Pedersen, S. S., Koch, C., Hoiby, N. & Rosendal, K. (1986). An epidemic spread of multi-resistant *Pseudomonas aeruginosa* in a cystic fibrosis centre. *Journal of Antimicrobial Chemotherapy* **17**, 505–16.

**37.** Denton, M. & Kerr, K. G. (1998). Microbiological and clinical aspects of infection associated with S*tenotrophomonas maltophilia. Clinical Microbiology Reviews* **11**, 57–80.

**38.** Nordmann, P. & Guibert, M. (1998). Extended-spectrum $\beta$-lactamases in *Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy* **42**, 128–31.

**39.** Coronado, V. G., Edwards, J. R., Culver, D. H. & Gaynes, R. P. (1995). Ciprofloxacin resistance among nosocomial *Pseudomonas aeruginosa* and *Staphylococcus aureus* in the United States. National Nosocomial Infections Surveillance (NNIS) System. *Infection Control and Hospital Epidemiology* **16**, 71–5.

**40.** Grassi, G. G. & Grassi, C. (1993). Cefepime: overview of activity *in vitro* and *in vivo*. *Journal of Antimicrobial Chemotherapy* **32**, *Suppl. B*, 87–94.

**41.** Edlund, C., Stark, C. & Nord, C. E. (1994). The relationship between an increase in $\beta$-lactamase activity after oral administration of three new cephalosporins and protection against intestinal ecological disturbances. *Journal of Antimicrobial Chemotherapy* **34**, 127–38.

**42.** George, R. C. & Uttley, A. H. C. (1989). Susceptibility of enterococci and epidemiology of enterococcal infection in the 1980s. *Epidemiology and Infection* **103**, 403–13.

**43.** Magnussen, C. R. & Cave, J. (1988). Nosocomial enterococcal infections: association with use of third-generation cephalosporin antibiotics. *American Journal of Infection Control* **16**, 241–5.

**44.** Guggenbichler, J. P., Allerberger F. J. & Dierich, M. (1986). Influence of cephalosporins III generation with varying biliary excretion on faecal flora and emergence of resistant bacteria during and after cessation of therapy. *Padiatrie und Padologie* **21**, 335–42.

**45.** Suppola, J. P., Volin, L., Valtonen, V. V. & Vaara, M. (1996). Overgrowth of *Enterococcus faecium* in the feces of patients with hematologic malignancies. *Clinical Infectious Diseases* **23**, 694–7.

**46.** Gentry, L. O. (1991). Bacterial resistance. *Orthopedic Clinics of North America* **22**, 379–88.

**47.** van der Waaij, D. (1987). Colonisation resistance of the digestive tract—mechanism and clinical consequences. *Nahrung* **31**, 507–17.

**48.** de Vries-Hospers, H. G., Tonk, R. H. & van der Waaij, D. (1991). Effect of intramuscular ceftriaxone on aerobic oral and faecal flora of 11 healthy volunteers. *Scandinavian Journal of Infectious Diseases* **23**, 625–33.

**49.** Manzella, J., Benenson, R., Pellerin, G., Kellogg, J., Bell, T., Robertson, M. & Pope, D. (2000). Choice of antibiotic and risk of colonization with vancomycin-resistant *Enterococcus* among patients admitted for treatment of community-acquired pneumonia. *Infection Control and Hospital Epidemiology* **21**, 789–91.

**50.** van der Waaij, D., Hofstra, H. & Wiegersma, N. (1982). Effect of beta-lactam antibiotics on the resistance of the digestive tract of mice to colonization. *Journal of Infectious Diseases* **146**, 417–22.

**51.** van der Waaij, D. (1985). Selective decontamination of the digestive tract with oral aztreonam and temocillin. *Reviews of Infectious Diseases* **7**, *Suppl. 4*, S628–34.

**52.** Woodford, N., Morrison, D., Johnson, P. & George, R. C. (1993). Antimicrobial resistance amongst enterococci isolated in the United Kingdom, a reference laboratory perspective. *Journal of Antimicrobial Chemotherapy* **32**, 344–6.

**53.** Uttley, A. H. C., Collins, C. H., Naidoo, J. & George, R. C. (1988). Vancomycin-resistant enterococci. *Lancet* **i**, 57–8.

**54.** Riley, T. V. (1996). *Clostridium difficile:* a high-cost nosocomial pathogen. *Culture* **17**, 2–4.

**55.** Ambrose, N. S., Johnson, M., Burdon, D. W. & Keighley, M. R. B. (1985). The influence of single dose intravenous antibiotics on faecal flora and emergence of *Clostridium difficile. Journal of Antimicrobial Chemotherapy* **15**, 319–26.

**56.** de Lalla, F., Privitera, G., Ortisi, G., Rizzardini, G., Santoro, D., Pagano, A. *et al.* (1989). Third generation cephalosporins as a risk factor for *Clostridium difficile*-associated disease: a four-year survey in a general hospital. *Journal of Antimicrobial Chemotherapy* **23**, 623–31.

**57.** Impallomeni, M., Galletly, N. P., Wort, J., Starr, J. M. & Rogers, T. R. (1995). Increased risk of diarrhoea caused by *Clostridium difficile* in elderly patients receiving cefotaxime. *British Medical Journal* **311**, 1345–6.

**58.** Chachaty, E., Depitre, C., Mario, N., Bourneix, C., Saulnier, P., Corthier, G. *et al.* (1992). Presence of *Clostridium difficile* and antibiotic and beta-lactamase activities in faeces of volunteers treated with oral cefixime, oral cefpodoxime proxetil, or placebo. *Antimicrobial Agents and Chemotherapy* **36**, 2009–13.

**59.** McFarland, L. V., Mulligan, M. E., Kwok, R. Y. & Stamm, N. E. (1989). Nosocomial acquisition of *Clostridium difficile* infection. *New England Journal of Medicine* **320**, 204–10.

**60.** Zadik, P. M. & Moore, A. P. (1998). Antimicrobial association of an outbreak of diarrhoea due to *Clostridium difficile. Journal of Hospital Infection* **39**, 189–93.

**61.** Wilcox, M. H., Cunniffe, J. G., Trundle, C. & Redpath, C. (1996). Financial burden of hospital-acquired infection. *Journal of Hospital Infection* **34**, 23–30.

**62.** Wilcox, M. H. & Smyth, E. T. M. (1998). Incidence and impact of *Clostridium difficile* infection in the UK, 1993–1996. *Journal of Hospital Infection* **39**, 181–7.

**63.** The British Thoracic Society. (1993). Guidelines for the management of community acquired pneumonia in adults admitted to hospital. *British Journal of Hospital Medicine* **49**, 346.

**64.** Spencer, R. C. (1998). The role of antimicrobial agents in the etiology of *Clostridium difficile*-associated disease. *Journal of Antimicrobial Chemotherapy* **41**, *Suppl. C*, 21–7.

**65.** Wort, S. J. & Rogers, T. R. (1998). Community-acquired pneumonia in elderly people. *British Medical Journal* **316**, 1690.

**66.** Stone, S. P., Beric, V., Quick, A., Balestrini, A. & Kibbler, C. (1998). The effect of an enhanced infection-control policy on the incidence of *Clostridium difficile* infection and methicillin-resistant *Staphylococcus aureus* colonization in acute elderly medical patients. *Age and Ageing* **27**, 561–8.

**67.** McNulty, C., Logan, M., Donald, I. P., Ennis, D., Taylor, D., Baldwin, R. N. *et al.* (1997). Successful control of *Clostridium difficile* infection in an elderly care unit through use of a restrictive antibiotic policy. *Journal of Antimicrobial Chemotherapy* **40**, 707–11.

**68.** Bross, J., Talbot, G. H., Maislin, G., Hurwitz, S. & Strom, B. L. (1989). Risk factors for nosocomial candidemia: a case–control study in adults without leukaemia. *American Journal of Medicine* **87**, 614–20.

**69.** Wey, S. B., Mori, M., Pfaller, M. A., Woolson, R. F. & Wenzel, R. P. (1989). Risk factors for hospital-acquired candidaemia. A matched case–control study. *Archives of Internal Medicine* **149**, 2349–53.

**70.** Kinsman, O. S. & Pitblado, K. (1989). *Candida albicans* gastrointestinal colonization and invasion in the mouse: effect of antibacterial dosing, antifungal therapy and immunosuppression. *Mycoses* **32**, 664–74.

**71.** Samonis, G., Dassiou, M. & Anastassiadou, H. (1994). Antibiotics affecting gastrointestinal colonization of mice by yeasts. *Journal of Chemotherapy* **6**, 50–2.

**72.** Thomakos, N., Maraki, S., Liakakos, T., Macheras, A., Kanakavi, S., Marinis, E. *et al.* (1998). Effect of cefamandole, cefuroxime and cefoxitin on yeast fecal flora of surgical patients. *Chemotherapy* **44**, 324–7.

**73.** Gubbins, P. O., McConnell, S. A. & Penzak S. R. (1999). Current management of funguria. *American Journal of Health Systems and Pharmacy* **56**, 1929–35.

**74.** Nassoura, Z., Ivatury, R. R., Simon, R. J., Jabbour, N. & Stahl, W. M. (1993). Candiduria as an early marker of disseminated infection in critically ill surgical patients: the role of fluconazole therapy. *Journal of Trauma* **35**, 290–5.

**75.** Wade, J. C. (1993). Epidemiology of *Candida* infections. In *Candidiasis*, 2nd edn, (Bodey, G. P., Ed.), pp. 85–107. Raven Press, New York.

**76.** Pfaller, M. A. (1995). Epidemiology of candidiasis. *Journal of Hospital Infection* **30**, *Suppl.*, 329–38.

**77.** Banerjee, S. N., Emori, T. G., Culver, D. H., Gaynes, R. P., Jarvis, W. R., Horan, T. *et al.* (1991). Secular trends in nosocomial primary bloodstream infections in the United States, 1980–1989. *American Journal of Medicine* **91**, *Suppl. 3B*, 86S–89S.

**78.** Pfaller, M. A., Messer, S. A., Hollis, R. J., Jones, R. N., Doern, G. V., Brandt, M. E. *et al.* (1999). Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. *Diagnostic Microbiology and Infectious Disease* **33**, 217–22.

**79.** Schaberg, D. R., Culver, D. H. & Gaynes, R. P. (1991). Major trends in the microbial etiology of nosocomial infection. *American Journal of Medicine* **91**, *Suppl. 3B*, 72S–75S.

**80.** Swartz, M.N. (1994). Hospital-acquired infections: diseases with increasingly limited therapies. *Proceedings of the National Academy of Sciences, USA* **91**, 2420–7.

**81.** Frank, M. O., Batteiger, B. E., Sorensen, S. J., Hartstein, A. I., Carr, J. A., McComb, J. S. *et al.* (1997). Decrease in expenditures and selected nosocomial infections following implementation of

an antimicrobial-prescribing improvement program. *Clinical Performance and Quality Health Care* **5**, 180–8.

**82.** Eggimann, P., Glauser, M. P., Auon, M., Meunier, F. & Cacandra, T. (1993). Cefepime monotherapy for the empirical treatment of fever in granulocytopenic cancer patients. *Journal of Antimicrobial Chemotherapy* **32**, *Suppl. B*, 151–63.

**83.** Gould, I. M. (1999). A review of the role of antibiotic policies in the control of antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **43**, 459–65.

**84.** Medeiros, A. A. (1984). Beta-lactamases. *British Medical Bulletin* **40**, 18–27.

**85.** Dancer, S. J., Shears, P. & Platt, D. J. (1997). Isolation and characterization of coliforms from glacial ice and water in Canada's High Arctic. *Journal of Applied Microbiology* **82**, 597–609.

**86.** Livermore, D. M. (1987). Clinical significance of beta-lactamase induction and stable depression in Gram-negative rods. *European Journal of Clinical Microbiology* **6**, 439–45.

**87.** Amyes, S. G. B. & Miles, R. S. (1998). Extended-spectrum $\beta$-lactamases: the role of inhibitors in therapy. *Journal of Antimicrobial Chemotherapy* **42**, 415–7.

**88.** Pechere, J. C. (1989). Emergence of resistance in Gram-negative bacilli during beta-lactam therapy: a challenge for the future. *European Journal of Cancer and Clinical Oncology* **25**, *Suppl. 2*, S17–23.

**89.** Bedenic, B. (1999). Molecular and genetic characterization of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae. ISID News* **5**, 4–7.

**90.** Amyes, S. G. B., Payne, D. J. & du Bois, S. K. (1992). Plasmid-mediated beta-lactamases responsible for penicillin and cephalosporin resistance. *Journal of Medical Microbiology* **36**, 6–9.

**91.** Jacoby, G. A. (1994). Genetics of extended-spectrum beta-lactamases. *European Journal of Clinical Microbiology and Infectious Diseases* **13**, *Suppl. 1*, S2–11.

**92.** Murray, P. R., Granich, G. G., Krogstad, D. J. & Niles, A. C. (1983). In-vivo selection of resistance to multiple cephalosporins by *Enterobacter cloacae. Journal of Infectious Diseases* **147**, 590.

**93.** Courcol, R. J., Pinkas, M. & Martin, G. R. (1989). A seven-year survey of antibiotic susceptibility and its relationship with usage. *Journal of Antimicrobial Chemotherapy* **23**, 441–521.

**94.** Mulgrave, L. (1991). The changing ecology of hospital bacteria and the selective role of cephalosporins. *Epidemiology and Infection* **106**, 121–32.

**95.** Sanders, W. E. & Sanders, C. C. (1988). Inducible $\beta$-lactamases: clinical and epidemiological implications for use of newer cephalosporins. *Reviews of Infectious Diseases* **10**, 830–8.

**96.** Chow, J., Fine, M. J., Shlaes, D. M., Quinn, J. P., Hooper, D. C., Johnson, M. P. *et al.* (1991). *Enterobacter* bacteremia: clinical feature and emergence of resistance during therapy. *Annals of Internal Medicine* **115**, 585–90.

**97.** Ballow, C. H. & Schentag, J. J. (1992). Trends in antibiotic utilization and bacterial resistance. Report of the National Nosocomial Resistance Surveillance Group. *Diagnostic Microbiology and Infectious Disease* **15**, 37S–42S.

**98.** Karas, J. A., Pillay, D. G., Muckart, D. & Sturm, A. W. (1996). Treatment failure due to extended-spectrum $\beta$-lactamases. *Journal of Antimicrobial Chemotherapy* **37**, 203–4.

**99.** Koontz, F. P., Jones, R. N. & Wenzel, R. P. (1990). Antibiotic resistance: experience at the University of Iona Hospitals and Clinics. In *Emerging Trends in Gram-Negative Resistance: A New Concern for Critical Care Medicine*, (Wayne, N. J., Ed.), pp. 16–9. Lederle Laboratories.

**100.** Rice, L. B., Willey, S. H., Papanicolaou, G. A., Medeiros, A. A., Eliopoulos, G. M., Moellering, R. C. *et al.* (1990). Outbreak of ceftazidime resistance caused by extended-spectrum $\beta$-lactamases at a Massachusetts chronic-care facility. *Antimicrobial Agents and Chemotherapy* **34**, 2193–9.

**101.** Meyer, K. S., Urban, C., Eagan, J. A., Berger, B. J. & Rahal, J. J. (1993). Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Annals of Internal Medicine* **119**, 353–8.

**102.** Quinn, J. P. (1994). Clinical significance of extended-spectrum $\beta$-lactamases. *European Journal of Clinical Microbiology and Infectious Diseases* **13**, *Suppl. 1*, S39–42.

**103.** Decker, M. D. & Schaffner, W. (1992). The relationship between the hospital and the community. In *Hospital Infections*, (Bennett, J. V. & Brachman, P. S., Eds), pp. 221–30. Little Brown, Boston.

**104.** Weinstein, R. A. (1987). Resistant bacteria and infection control in the nursing home and hospital. *Bulletin of the New York Academy of Medicine* **63**, 337–44.

**105.** Appelbaum, P. C. (1992). Antimicrobial resistance to *Streptococcus pneumoniae*: an overview. *Clinical Infectious Diseases* **15**, 77–83.

**106.** Doern, G. V., Brueggemann, A., Holley, H. P. & Rauch, A. M. (1996). Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during the winter months of 1994–1995: results of a thirty-centre national surveillance study. *Antimicrobial Agents and Chemotherapy* **40**, 1208–13.

**107.** Jacobs, M. R., Koornhof, H. J., Robins-Browne, R. M., Stevenson, C. M., Vermaak, Z. A., Freiman, I. *et al.* (1978). Emergence of multiply resistant pneumococci. *New England Journal of Medicine* **299**, 735–40.

**108.** Negri, M. C., Morosini, M. I., Loza, E. & Baquero, F. (1994). In vitro selective antibiotic concentrations of beta-lactams for penicillin-resistant *Streptococcus pneumoniae* populations. *Antimicrobial Agents and Chemotherapy* **38**, 122–5.

**109.** Goldstein, F. W. (1999). Penicillin-resistant *Streptococcus pneumoniae:* selection by $\beta$-lactam and non $\beta$-lactam antibiotics. *Journal of Antimicrobial Chemotherapy* **44**, 141–4.

**110.** Allen, K. D. & Anson, J. J. (1996). Prevalence of antibiotic resistance in pneumococci is higher in Merseyside. *British Medical Journal* **313**, 820.

**111.** Nava, J. M., Bella, F., Garau, J., Lite, J., Morera, M.-A., Marti, C. *et al.* (1994). Predictive factors for invasive disease due to penicillin resistant *Streptococcus pneumoniae*: a population based study. *Clinical Infectious Diseases* **19**, 884–90.

**112.** Baquero, F., Martinez-Beltran, J. & Loza, E. (1991). A review of antibiotic resistant patterns of *S. pneumoniae* in Europe. *Journal of Antimicrobial Chemotherapy* **28**, *Suppl. C*, 31–8.

**113.** Coffey, T. J., Dowson, C. G., Daniels, M. & Spratt, B. G. (1995). Genetics and molecular biology of $\beta$-lactam resistant pneumococci. *Microbial Drug Resistance* **1**, 29–34.

**114.** Verhaergen, J. & Verbist, L. (1998). In-vitro activity of 21 $\beta$-lactam antibiotics against penicillin-susceptible and penicillin-resistant *Streptococcus pneumoniae. Journal of Antimicrobial Chemotherapy* **41**, 381–5.

**115.** Perez-Trallero, E., Alkorta, M., Garcia-Arenzana, J. M., Iturzaeta, A. & Gomariz, M. (1998). In-vitro, in-vivo and ex-vivo studies with oral $\beta$-lactams against *Streptococcus pneumoniae*. *Journal of Antimicrobial Chemotherapy* **41**, 629–34.

**116.** Baquero, F. (1996). Trends in antibiotic resistance of respiratory pathogens: an analysis and commentary on collaborative surveillance study. *Journal of Antimicrobial Chemotherapy* **38**, *Suppl. A*, 117–32.

**117.** Cohen, R., Navel, M., Grumberg, J., Narcy, P., Boucherat, M., Geslin, P. *et al.* (1997). Single dose ceftriaxone (CRO) for acute otitis media in areas with high resistance to *Streptococcus pneumoniae.* In *Program and Abstracts of the Thirty-seventh Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, 1997*. Abstract LM-34, p. 370. American Society for Microbiology, Washington, DC.

**118.** Arnold, K. E., Leggiadro, R. J., Breiman, R. F., Lipman, H. B., Schwartz, B., Appleton, M. A. *et al.* (1996). Risk factors for carriage of drug resistant S*treptococcus pneumoniae* among children in Memphis, Tennessee. *Journal of Pediatrics* **128**, 757–64.

**119.** Ford, K. L., Mason, E. O., Kaplan, S. L., Lamberth, L. B. & Tillman, J. (1991). Factors associated with middle ear isolates of S*treptococcus pneumoniae* resistant to penicillin in a children's hospital. *Journal of Pediatrics* **119**, 941–4.

**120.** Tan, T. Q., Mason, E. O. & Kaplan, S. L. (1993). Penicillin-resistant systemic pneumococcal infections in children: a retrospective case-control study. *Pediatrics* **92**, 761–7.

**121.** Renneberg, J., Niemann, L. L. & Gutschik, E. (1997). Antimicrobial susceptibility of 278 streptococcal blood isolates to seven antimicrobial agents. *Journal of Antimicrobial Chemotherapy* **39**, 135–40.

**122.** Marchese, A., Debbia, E. A., Arvigo, A., Pesce, A. & Schito, G. C. (1995). Susceptibility of *Streptococcus pneumoniae* strains isolated in Italy to penicillin and ten other antibiotics. *Journal of Antimicrobial Chemotherapy* **36**, 833–7.

**123.** Halls, G. A. (1993). The management of infections and antibiotic therapy: a European survey. *Journal of Antimicrobial Chemotherapy* **31**, 985–1000.

**124.** Dabernat, H., Geslin, P., Megraud, F., Begue, P., Boulesteix, J., Dubreuil, C. *et al.* (1998). Effects of cefixime or co-amoxiclav treatment on nasopharyngeal carriage of *Streptococcus pneumoniae* and *Haemophilus influenzae* in children with acute otitis media. *Journal of Antimicrobial Chemotherapy* **41**, 253–8.

**125.** Sifaoui, F., Kitzis, M. D. & Gutmann, L. (1996). In vitro selection of one-step mutants of *S. pneumoniae* resistant to different oral $\beta$-lactams antibiotics is associated with alterations of PBP2x. *Antimicrobial Agents and Chemotherapy* **40**, 152–6.

**126.** Bauernfeind, A., Jungwirth, R., Schweighart, S. & Theopold, M. (1990). Antibakterielle Aktivitat und $\beta$-Laktamase – Stabilitat von elf Oralcephalosporinen. *Infection* **18**, *Suppl. 3*, S155–67.

**127.** Goldsmith, C. E., Moore, J. E. & Murphy, P. G. (1996). Prevalence of antibiotic resistance in pneumococci is higher in Northern Ireland. *British Medical Journal* **313**, 820.

**128.** Granizo, J. J., Aguilar, L. L., Casal, J., Garcia-Rey, C., Dal-Re, R. & Baquero, F. (2000). *Streptococcus pneumoniae* resistance to erythromycin and penicillin in relation to macrolides and $\beta$-lactam consumption in Spain (1979–1997). *Journal of Antimicrobial Chemotherapy* **46**, 767–73.

**129.** Arason, V. A., Kristinsson, K. G., Sigurdsson, J. A., Stefansdotir, G., Molstad, S. & Gudmundsson, S. (1996). Do antimicrobials increase the drainage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. *British Medical Journal* **813**, 387–91.

**130.** Gehanno, P., N'Guyen, L., Derriennic, M., Pichon, F., Goehrs, J. M. & Berche, P. (1998). Pathogens isolated during treatment failures in otitis. *Pediatric Infectious Diseases Journal* **17**, 885–90.

**131.** House of Lords Select Committee on Science and Technology. (1998). *Resistance to Antibiotics and Other Antimicrobial Agents*. Stationery Office, London.

**132.** Anonymous. (2000). *Staphylococcus aureus* bacteraemia: England and Wales. *CDR Communicable Disease Report Weekly* **10**, 143–4.

**133.** Jevons, M. P. (1961). Celbenin-resistant staphylococci. *British Medical Journal* **1**, 124–5.

**134.** Batchelor, F. R. (2000). Post penicillin antibiotics: from acceptance to resistance? In *Wellcome Witnesses to Twentieth Century Medicine*, 6th edn, (Tansey, E. M. & Reynolds, L. A., Eds), pp. 54. The Wellcome Trust, London.

**135.** Ayliffe, G. A. J. (1997). The progressive intercontinental spread of MRSA. C*linical Infectious Diseases* **24**, *Suppl. 1*, S74–9.

**136.** Dacre, J., Emmerson, A. M. & Jenner, E. A. (1986). Gentamicin–methicillin-resistant *Staphylococcus aureus*: epidemiology and containment of an outbreak. *Journal of Hospital Infection* **7**, 130–6.

**137.** Yamaguchi, K. & Ohno, A. (1992). Consideration on MRSA infections in relation to modern chemotherapy. *Nihon Rinsho* **50**, 923–31.

**138.** Ubukata, K., Nonoguchi, R., Dong Song, M., Matsuhashi, M. & Konno, M. (1990). Expression and inducibility of methicillin resistance encoded by a *mecA* gene in staphylococci. In *Molecular Biology of the Staphylococci*, (Novick, R. P., Ed.), pp. 471–89. VCH Publishers, New York.

**139.** Fukatsu, K., Saito, H., Matsuda, T., Ikeda, S., Furukawa, S. & Muto, T. (1997). Influences of type and duration of antimicrobial prophylaxis on an outbreak of methicillin-resistant *Staphylococcus aureus* and on the incidence of wound infection. *Archives of Surgery* **132**, 1320–5.

**140.** Casewell, M. W. & Hill, R. L. R. (1986). The carrier state: methicillin resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **18**, 1–12.

**141.** Voss, A., Milatovic, D., Wallrauch-Schwarz, C., Rosdahl, V. T. & Braveny, I. (1994). Methicillin-resistant *Staphylococcus aureus* in Europe. *European Journal of Clinical Microbiology and Infectious Diseases* **13**, 50–5.

**142.** Voss, A. (1996). *Staphylococcus aureus.* Pan-European antibiotic resistance and infection control. *Chemotherapie Journal* **5**, 5–6.

**143.** Saravolatz, L. D., Pohlod, D. & Arking, L. (1982). Community acquired MRSA infections: a new source for nosocomial outbreaks. *Annals of Internal Medicine* **97**, 325–9.

**144.** Chambers, H. F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clinical Microbiology Reviews* **10**, 781–91.

**145.** Okonogi, K. (1990). Mechanism of $\beta$-lactam-resistance in MRSA. *Japanese Journal of Clinical Pathology* **38**, 983–9.

**146.** Okonogi, K., Noji, Y., Kondo, M., Imada, A. & Yakota, T. (1989). Emergence of methicillin-resistant clones from cephamycin-

resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **24**, 637–45.

**147.** Washio, M. (1997). Risk factors for methicillin-resistant *S. aureus* (MRSA) infection in a Japanese elderly care nursing home. *Epidemiology and Infection* **119**, 285.

**148.** Crossley, K., Loesch, D., Landesman, B., Mead, K., Chern, M. & Strate, R. (1979). An outbreak of infections caused by strains of *Staphylococcus aureus* resistant to methicillin and aminoglycosides. *Journal of Infectious Diseases* **139**, 273–9.

**149.** Washio, M., Mizoue, T., Kajioka, T., Yoshimitsu, T., Okayama, M., Hamada, T. *et al*. (1997). Risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) infection in a Japanese geriatric hospital. *Public Health* **111**, 187–90.

**150.** Peacock, J. E., Marsik, F. J. & Wenzel, R. P. (1980). Methicillin-resistant *Staphylococcus aureus:* introduction and spread within a hospital. *Annals of Internal Medicine* **93**, 526–32.

**151.** Schentag, J. J., Hyatt, J. M., Carr, J. R., Paladino, J. A., Birmingham, M. C., Zimmer, G. S. *et al.* (1998). Genesis of methicillin-resistant *Staphylococcus aureus* (MRSA), how treatment of MRSA infections has selected for vancomycin-resistant *Enterococcus faecium*, and the importance of antibiotic management and infection control. *Clinical Infectious Diseases* **26**, 1204–14.

**152.** Kind, A. C., Kestle, D. G., Standiford, H. C., Freeman, P. & Kirby, W. M. M. (1968). Development of staphylococci cross-resistant to cephalexin and methicillin. *Antimicrobial Agents and Chemotherapy* **8**, 405–9.

**153.** Wiedemann, B. (1986). Selection of $\beta$-lactamase producers during cephalosporin and penicillin therapy. *Scandinavian Journal of Infectious Diseases* **49**, *Suppl*., 100–5.

**154.** Noble, W. C. & Naidoo, J. (1978). Evolution of antibiotic resistance in *Staphylococcus aureus*: the role of the skin. *British Journal of Dermatology* **98**, 481–9.

**155.** Forbes, B. A. & Schaberg, D. R. (1983). Transfer of resistant plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugate exchange of resistance. *Journal of Bacteriology* **153**, 627–6.

**156.** Archer, G. L., Niemeyer, D. M., Thanassi, J. A. & Pucci, M. J. (1994). Dissemination among staphylococci of DNA sequences associated with methicillin resistance. *Antimicrobial Agents and Chemotherapy* **38**, 447–54.

**157.** Hughes, W. T., Armstrong, D., Bodey, G. & The IDSA Working Group. (1990). Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. *Journal of Infectious Diseases* **161**, 381–96.

**158.** Mannion, P. T., Thom, B. T., Reynolds, C. S. & Strachan, C. J. L. (1989). The acquisition of antibiotic resistant coagulase-negative staphylococci by aortic graft recipients. *Journal of Hospital Infection* **14**, 313–23.

**159.** Asensio, A., Guerrero, A., Quereda, C., Lizan, M. & Martinez-Ferrer, M. (1996). Colonization and infection with methicillin-resistant *Staphylococcus aureus:* associated factors and eradication. *Infection Control and Hospital Epidemiology* **17**, 20–8.

**160.** Hill, D. A., Herford, T. & Parratt, D. (1998). Antibiotic usage and methicillin-resistant *Staphylococci aureus:* an analysis of casualty. *Journal of Antimicrobial Chemotherapy* **42**, 676–7.

**161.** Smith, D. W. (1999). Decreased antimicrobial resistance after changes in antibiotic use. *Pharmacotherapy* **19**, 129S–132S.

**162.** Wagenvoort, J. H. T. (2000). Dutch measures to control MRSA and the expanding European Union. *Eurosurveillance* **5**, 26–8.

**163.** Anonymous. (1999). Four pediatric deaths from community-acquired MRSA – Minnesota and North Dakota, 1997–1999. *MMR Morbidity and Mortality Weekly Report* **48**, 707–10.

**164.** Acar, J. F., Courvalin, P. & Chabbert, Y. A. (1970). Methicillin-resistant staphylococcaemia: bacteriological failure of treatment with cephalosporins. *Antimicrobial Agents and Chemotherapy* **10**, 280–5.

**165.** Collins, J. K., Mader, J. T. & Kelly, M. T. (1983). Resistance of methicillin-resistant *Staphylococcus aureus* to third-generation cephalosporins. *Journal of Infectious Diseases* **147**, 591.

**166.** Soussy, C. J., Deforges, L., Le Van Thoi, J., Chanal, M., Sirot, D., Acar, J. F. *et al*. (1985). In vitro activity of ceftriaxone on hospital bacteria. Results of a multicenter study. *Pathologie Biologie* **33**, 469–72.

**167.** Kernodle, D. S., Classen, D. C., Stratton, D. W. & Kaiser, A. B. (1998). Association of borderline oxacillin-susceptible strains of *Staphylococcus aureus* with surgical wound infections. *Journal of Clinical Microbiology* **36**, 219–22.

**168.** Stewart, B., Hall, L., Duke, B. & Ball, D. (1997). Vancomycin-dependent enterococci: curious phenomenon or serious threat? *Journal of Antimicrobial Chemotherapy* **40**, 734–5.

**169.** Spera, R. V. & Farber, B. F. (1994). Multi drug resistant *Enterococcus faecium.* An untreatable nosocomial pathogen. *Drug* **48**, 678–88.

**170.** Bradley, S. J., Wilson, A. L. T., Allen, M. C., Sher, H. A., Goldstone, A. H. & Scott, G. M. (1999). The control of hyperendemic glycopeptide-resistant *Enterococcus* spp. on a haematology unit by changing antibiotic usage. *Journal of Antimicrobial Chemotherapy* **43**, 261–6.

**171.** Fraise, A. P. (1996). The treatment and control of vancomycin-resistant enterococci. *Journal of Antimicrobial Chemotherapy* **38**, 753–6.

**172.** Wade, J. J. (1995). The emergence of *Enterococcus faecium* resistant to glycopeptides and other standard agents – a preliminary report. *Journal of Hospital Infection* **30**, *Suppl*., 483–93.

**173.** Chadwick, P. R., Chadwick, C. D. & Oppenheim, B. A. (1996). Report of a meeting on the epidemiology and control of glycopeptide-resistant enterococci. *Journal of Hospital Infection* **33**, 83–92.

**174.** Van den Bogaard, A. E., Mertens, P., London, N. H. & Stobberingh, E. E. (1997). High prevalence of colonization with vancomycin- and pristinamycin-resistant enterococci in healthy humans and pigs in The Netherlands: is the addition of antibiotics to animal feeds to blame? *Journal of Antimicrobial Chemotherapy* **40**, 454–6.

**175.** Witte, W. & Klare, I. (1995). Glycopeptide-resistant *Enterococcus faecium* outside hospitals: a commentary. *Microbial Drug Resistance* **1**, 259–63.

**176.** Lam, S., Singer, C., Tucci, V., Morthland, V. H., Pfaller, M. A. & Isenverg, H. D. (1995). The challenge of vancomycin-resistant enterococci: a clinical and epidemiologic study. *American Journal of Infection Control* **23**, 170–80.

**177.** Watanakunakorn, C. (1982). Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. *Annals of Internal Medicine* **97**, 376–8.

**178.** Tedesco, F., Markham, R., Gurwith, M., Christie, D. & Bartlett, J. G. (1978). Oral vancomycin for antibiotic-associated pseudomembranous colitis. *Lancet* **ii**, 226–8.

**179.** Taylor, M. E., Oppenheim, B. A., Chadwick, P. R., Weston, D., Palepou, M. F., Woodford, N. *et al.* (1999). Detection of glycopeptide-resistant enterococci in routine diagnostic faeces specimens. *Journal of Hospital Infection* **43**, 25–32.

**180.** Dukta-Malen, S., Leclerq, R., Coutant, V., Duval, J. & Courvalin, P. (1990). Phenotypic and genotypic heterogeneity of glycopeptide resistance determinants in gram-positive bacteria. *Antimicrobial Agents and Chemotherapy* **34**, 1875–9.

**181.** Kjerulf, A., Pallesen, L. & Westh, H. (1996). Vancomycin-resistant enterococci at a large university hospital in Denmark. *APMIS* **104**, 475–9.

**182.** Tucci, V., Haran, M. A. & Isenberg, H. D. (1997). Epidemiology and control of vancomycin-resistant enterococci in an adult and children's hospital. *American Journal of Infection Control* **25**, 371–6.

**183.** Morgan, A. S., Brennan, P. J. & Fishman, N. O. (1997). Impact of a vancomycin restriction policy on use and cost of vancomycin and incidence of vancomycin-resistant *Enterococcus*. *Annals of Pharmacotherapy* **31**, 970–3.

**184.** Morris, J. G., Shay, D. K., Hebden, J. N., McCarter, R. J., Perdue, B. E., Jarvis, W. *et al.* (1995). Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. *Annals of Internal Medicine* **123**, 250–9.

**185.** Wendt, C., Wiesenthal, B., Dietz, E. & Ruden, H. (1998). Vancomycin-resistant and vancomycin-susceptible enterococci on dry surfaces. *Journal of Clinical Microbiology* **36**, 3734–6.

**186.** Dancer, S. J. (1999). Mopping up hospital infection. *Journal of Hospital Infection* **43**, 85–100.

**187.** Kawalek, M., Gniadkowski, M. & Hryniewicz, W. (2000). Outbreak of vancomycin-resistant enterococci in a hospital in Gdansk, Poland, due to horizontal transfer of different *Tn*1546-like transposon variants and clonal spread of several strains. *Journal of Clinical Microbiology* **38**, 3317–22.

**188.** Rozalska, M., Szewczyk, E. & Piotrowski, A. (1993). Bacterial flora of the hospital environment: identification, susceptibility to disinfecting agents, antibiotics and chemotherapeutics. *Medycyna Doswiadczalna i Mikrobiologia* **45**, 373–8.

**189.** Noble, W. C. & Virani, Z. (1992). Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiology Letters* **93**, 195–8.

**190.** Leclercq, R., Derlot, E., Weber, M., Duval, J. & Courvalin, P. (1989). Transferable vancomycin and teicoplanin resistance in *Enterococcus faecium*. *Antimicrobial Agents and Chemotherapy* **33**, 10–5.

**191.** Boyle-Vavra, S., Daum, R. S., Labischinski, H. & Hiramatsu, K. (1998). Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant S*taphylococcus aureus* clinical strains Mu3 and Mu50. *Journal of Antimicrobial Chemotherapy* **42**, 199–209.

**192.** Hanberger, H., Nilsson, L. E., Nilsson, M. & Maller, R. (1991). Post-antibiotic effect of $\beta$-lactam antibiotics on gram-negative bacteria in relation to morphology, initial killing and MIC. *European Journal of Clinical Microbiology and Infectious Diseases* **10**, 927–34.

**193.** Rolinson, G. N. (1998). Forty years of $\beta$-lactam research. *Journal of Antimicrobial Chemotherapy* **41**, 589–603.

**194.** Rolinson, G. N. (1980). Effect of $\beta$-lactam antibiotics on bacterial cell growth rate. *Journal of General Microbiology* **120**, 317–23.

**195.** Gutmann, l., Vincent, S., Billot-Klein, D., Acar, J. F., Mrena, E. & Williamson, R. (1986). Involvement of penicillin-binding protein 2 with other penicillin-binding proteins in lysis of *Escherichia coli* by some $\beta$-lactam antibiotics alone and in synergistic lytic effect of amdinocillin (mecillinam). *Antimicrobial Agents and Chemotherapy* **30**, 906–12.

**196.** Gould, I. & Mackenzie, F. M. (1997). The response of Enterobacteriaceae to $\beta$-lactam antibiotics—'round forms, filaments and the root of all evil'. *Journal of Antimicrobial Chemotherapy* **40**, 495–9.

**197.** Hurley, J. C. (1992). Antibiotic induced release of endotoxin: a reappraisal. *Clinical Infectious Diseases* **15**, 840–54.

**198.** Dofferhoff, A. S. M., Nijland, J. H., de Vries-Hospers, H. G., Mulder, P. O. M., Weits, J. & Bom, V. J. J. (1991). Effects of different types and combinations of antimicrobial agents on endotoxin release from Gram-negative bacteria: an in-vitro and in-vivo study. *Scandinavian Journal of Infectious Diseases* **23**, 745–54.

**199.** Geddes, A. M. & Gould, I. M. (1993). The effects of plasmid-mediated cephalosporinases and endotoxins on septic patients. *Serodiagnosis, Immunotherapy, and Infectious Disease* **5**, 201–3.

**200.** Shenep, J. L., Flynn, P. M., Barrett, F. F., Stidham, G. L. & Westenkirchner, D. F. (1988). Serial quantitation of endotoxemia and bacteremia during therapy for Gram-negative sepsis. *Journal of Infectious Diseases* **157**, 565–8.

**201.** Periti, P. & Mazzei, T. (1999). New criteria for selecting the proper antimicrobial chemotherapy for severe sepsis and septic shock. *International Journal of Antimicrobial Agents* **12**, 97–105.

**202.** Finch, R. G. (1998). Antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **42**, 125–8.

**203.** Ruiz Bremon, A., Ruiz-Tovar, M., Perez Gorricho, B., Diaz de Torres, P. & Lopez Rodriguez, R. (2000). Non-hospital consumption of antibiotics in Spain: 1987–1997. *Journal of Antimicrobial Chemotherapy* **45**, 395–400.

**204.** McGowan, J. E. (1994). Do intensive hospital antibiotic control programs prevent the spread of antibiotic resistance? *Infection Control and Hospital Epidemiology* **15**, 478–83.

**205.** Shales, D. M., Gerding, D. N., John, J. F., Craig, W. A., Bornstein, D. L., Duncan, R. A. *et al.* (1997). Guidelines for the prevention of antimicrobial resistance in hospitals. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance. *Infection Control and Hospital Epidemiology* **18**, 275–91.

**206.** Kolmos, H. J. (1999). Interaction between the microbiology laboratory and clinician: what the microbiologist can provide. *Journal of Hospital Infection*, **43**, *Suppl.*, S285–91.

**207.** Beam, T. R. (1988). Recent advances in curtailing costs of antimicrobial agents. *The Antimicrobic Newsletter* **5**, 17–21.

**208.** Gould, I. M. (1988). Control of antibiotic use in the United Kingdom. *Journal of Antimicrobial Chemotherapy* **22**, 395–401.

**209.** Leistevuo, T., Osterblad, M., Toivonen, P., Kahra, A., Lehtonen, A. & Huovinen, P. (1996). Colonization of resistant faecal aerobic Gram-negative bacilli among geriatric patients in hospital and the community. *Journal of Antimicrobial Chemotherapy* **37**, 169–73.

**210.** Loefler, I. J. P. (1996). Microbes, chemotherapy, evolution, and folly. *Lancet* **348**, 1703–4.

**211.** Davey, P., Hudson, S., Ridgway, G. & Reeves, D. (1993). A survey of undergraduate and continuing medical education and about antimicrobial chemotherapy in the United Kingdom. *British Journal of Clinical Pharmacology* **36**, 511–9.

**212.** Neu, H. C. (1990). Third generation cephalosporins: safety profiles after 10 years of clinical use. *Journal of Clinical Pharmocology* **30**, 396–403.

**213.** Sanderson, P. J. (1993). Antimicrobiological prophylaxis in surgery; microbiological factors. *Journal of Antimicrobial Chemotherapy* **31**, *Suppl. B*, 1–9.

**214.** Livermore, D. (2000). Epidemiology of antibiotic resistance. *Intensive Care Medicine* **26**, *Suppl. 1*, S14–21.

**215.** Amyes, S. G. B. & Thomson, C. J. (1995). Antibiotic resistance in the ICU; the eve of destruction. *British Journal of Intensive Care* **5**, 263–7.

**216.** Desowitz, R. S. (1987). *New Guinea Tapeworms and Jewish Grandmothers.* W. W. Norton & Co., New York, London.

**217.** Bernard, G. R., Vincent, J. L., Laterre, P. F., LaRosa, S. P., Dhainaut, J. F., Lopez-Rodriguez, A. *et al*. (2001). Efficacy and safety of recombinant human activated protein C for severe sepsis. *New England Journal of Medicine* **344**, 699–709.

**218.** Vincent, J. L. (2000). Afelimomab. *International Journal of Clinical Practice* **54**, 190–3.