UNTREATABLE INFECTIONS? -THE CHALLENGE OF THE 21st CENTURY

Christopher M Parry

Wellcome Trust Clinical Research Unit, Center for Tropical Diseases, Ho Chi Minh City, Vietnam; Center for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, United Kingdom

Abstract. The triumph of antibiotics over bacterial pathgogens that has occurred in the latter half of this century looks increasingly threatened as we approach the new millennium. Increasing resistance in important pathogens such as *Mycobacterium tuberculosis, Shigella,* and *Streptococcus pneumoniae* threatens the lives of millions. The increasing problems with drug resistance in *C.diphtheriae, Salmonella typhi* and the pneumococcus in Vietnam are presented as examples of the challenge confronting tropical countries.

INTRODUCTION

At the beginning of the 20th Century infections were a major cause of morbidity and mortality in humans. Diseases such as pneumococcal pneumonia, cerebrospinal fever, typhoid fever, diphtheria, dysentery, puerperal sepsis, tuberculosis and malaria were common and often lethal. The introduction of antimicrobial and chemotherepeutic agents in the 1940s and 1950s had a dramatic impact, improving the outcome and reducing the spread of many of these infectious diseases. As we now approach the end of this century the increase in resistance to these antimicrobial agents threatens to reverse those achievements (Neu 1992; Shears, 1993; Tomasz, 1994). Antimicrobial resistance has led to increases in morbidity and mortality and increases in the cost of health care which threaten to become unaffordable in poor countries. Outbreaks due to increased transmission of resistant pathogens following inadequate treatment also occur. Resistance is an important problem both in the hospital and the community, in bacteria, viruses, fungi and parasites. Some hospital pathogens have recently emerged which are resistant to nearly all antibiotics.

This article will focus on the impending crisis of antibiotic resistance in bacterial pathogens, describing some antibiotic resistance problems of community acquired pathogens in Vietnam, reviewing the mechanism, molecular basis, and spread of resistance in bacteria and the steps that could be taken to prevent the "post-antimicrobial era".

Correspondence: Wellcome Trust Clinical Research Unit, Center for Tropical Diseases, 190 Ben Ham Tu, District 5, Ho Chi Minh City, Vietnam.
Tel: 00848-8353954; Fax: 00848-8353940; E-mail: cparry@hcm.vnn.vn

MULTIPLE-ANTIBIOTIC RESISTANT BACTERIA IN VIETNAM

Antibiotic resistance is rising rapidly in many bacterial pathogens in Vietnam. Recent experience with multiple-antibiotic resistant *Salmonella typhi, Streptococcus pneumoniae* and *Corynebacterium diphtheriae* illustrate the emerging problems of antibiotic resistance in community acquired pathogens which are typical of many countries.

Salmonella typhi

At the turn of the century typhoid fever was a prolonged, debilitating illness with a mortality of 30%. In the late 1940s Woodward and colleagues discovered the beneficial effects of chloramphenicol in typhoid fever (Woodward *et al,* 1948). With chloramphenicol, the fever fell rapidly and the mortality was reduced from 30% to less than 1%. Despite problems with relapses and persistent fecal carriage, the drug revolutionized the treatment of typhoid fever and became the standard treatment for the next 25 years. In 1972 however, there was an extensive epidemic of chloramphenicol resistant typhoid fever in Mexico affecting more than 10,000 people (Olarte and Galindo, 1973). Chloramphenicol resistant typhoid subsequently emerged in

other countries in South America and extensively in Asia. These strains were resistant to chloramphenicol, sulphonamides, aminoglycosides and tetracycline. Amoxycillin and co-trimoxazole were found to be effective alternatives (Butler *et al,* 1977). Chloramphenicol resistance in Vietnam and Thailand reached levels of 75-80% in the late 1970s but fell to 45% a decade later as other drugs were used for treatment (Butler *et al,* 1977; Hoa *et al* 1992; Thisyakorn *et al,* 1987).

The first multidrug-resistant (MDR) strains emerged in China in 1987 and were simultaneously resistant to chloramphenicol, ampicillin, trimethoprim, sulphonamides, tetracycline and aminoglycosides. They have since spread throughout the Asia region becoming endemic in many areas and causing localized epidemics in India and Vietnam (Anand *et al,* 1990; Mirza *et al,* 1996; Hien *et al,* 1995). In the Middle East MDR strains have been seen in expatriate workers from Asia. Elsewhere in Africa and South and Central America, although sporadic cases have been reported, MDR strains have not been a major problem. MDR *S.typhi* began to appear in Vietnam during 1991. Fig 1 shows the number of blood culture confirmed cases of typhoid fever seen at the Center for Tropical Diseases, Ho Chi Minh City, an infectious disease referral center for the city and southern provinces. The proportion of MDR isolates increased from 27% in 1992 to 89% in 1995.

Clinical trials in Vietnam and other centers have shown that MDR *S.typhi* can be effectively treated with fluoroquinolones and third generation cephalosporins. The fluoroquinolones are clinically superior (White and Parry, 1996) and effective and safe in courses as short as 2-5 days provided the strains are fully fluoroquinolone sensitive. Unfortunately a second epidemic of drug resistant typhoid fever is now emerging in Vietnam and the Indian subcontinent, that of reduced susceptibility to the fluoroquinolone antibiotics (Jesudason *et al,* 1996; Wain *et al,* 1997). Although the MIC of these strains are below the fluoroquinolone breakpoint, they are less susceptible to fluoroquinolones than the fully sensitive strains, are resistant to the quinolone nalidixic acid (Wain *et al,* 1997). In Vietnam these strains first appeared in 1993, increasing showly until 1997 (Fig 1). In the last months of 1997 an outbreak in Ho Chi Minh City took the percentage of isolates from blood culture at our Center to 80% (Parry *et al,* 1998).

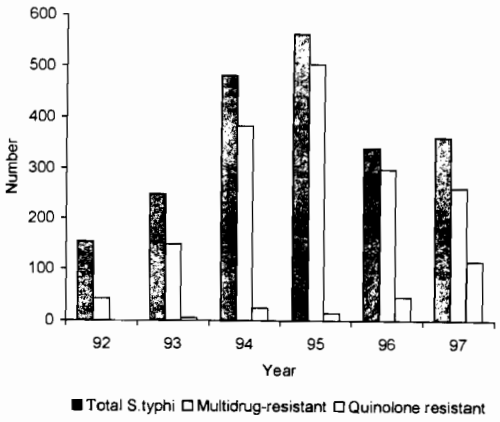

Fig 1–*Salmonella typhi* isolated from blood cultures at the Center for Tropical Diseases, Ho Chi Minh City, Vietnam.
■ Total *S.typhi* □Multidrug-resistant □Quinolone resistant

Short course ofloxacin treatment of uncomplicated typhoid fever is successful in 98% of patients with a quinolone sensitive strain, but only 50% in those with a quinolone resistant isolate (Wain *et al,* 1997). Increasing the duration of treatment to seven days still results in prolonged fever clearances, failure rates of up to 10% and relapse rates of 3% (unpublished observations). Quinolone resistance in Vietnam is not restricted to *S.typhi*. Fully fluoroquinolone resistant isolates of *E.coli* and *Klebsiella* sp isolated from blood cultures at this center have increased from 2% in 1993 to 8% in 1997 and in urine from 0% in 1993 to 24% in 1997 (unpublished observations).

A rough calculation of the average cost of admission (including bed fees, investigations and the cost of treatment) for a patient infected with nalidixic acid resistant typhoid is US $ 50 compared with US $ 22 for a patient infected with a sensitive strain. Treatment of these bacteria with reduced fluoro- quinolone susceptibility is an acute problem as the third generation cephalosporins and carbapenems are unaffordable for many people.

Streptococcus pneumoniae

The pneumococcus is a major global pathogen causing an estimated 3 million to 5 million deaths per year. The introduction of penicillin in the 1940s had a tremendous impact on reducing the mortality

of pneumococcal disease. The emergence of penicillin resistance in pneumococci was therefore a worrying development. Low level resistance appeared in Papua New Guinea in 1967 and high level resistance in South Africa in 1977. Penicillin resistant pneumococci have now appeared in many areas of the world and in some areas have reached high levels. Recent studies from Asia have shown high levels of penicillin resistane (Lee *et al,* 1995; Yoshida *et al,* 1995). Penicillin resistance is also emerging in Vietnam. In the years 1993-1995 10% of invasive isolates at the Ceter for Tropical Diseases were penicillin resistant. In 1996-1997 this had risen to 56% (p=0.03) (Parry *et al,* 1997b). Although the resistance is still low level,treatment failures have occurred with third generation cephalosporins. If high level penicillin resistant isolates appear this may necessitate changes in the empirical antibiotic choice for the treatment of meningitis. For many patients vancomycin is prohibitively expensive.

High levels of penicillin resistance have also been found in pneumococci carried in the nasopharynx of local children. In a study of almost 500 *Streptococcus pneumoniae* isolated from the nose of healthy schoolchildren the level of penicillin resistance varied from 17% in rural chidren to 87% in the urban children (Parry *et al,* 1997a). Furthermore resistance was present to other commonly used antibiotics including tetracycline, cotrimoxazole, chloramphenicol and erythromycin. This reflects a significant reservoir of resistance genes within pneumococci in the community that is likely to subsequently appear in invasive strains.

Corynebacterium diphtheriae

The incidence of diphtheria has declined in the last ten years in Vietnam with the more widespread uptake of vaccination. However about 50 cases a year are still seen at the Center for Tropical Diseases, Ho Chi Minh City. The early administration of antitoxin is the key component of the effective management of diphtheria. Antibiotic therapy is important to rapidly eradicate the organism from the throat, eliminating further toxin production, and to prevent the spread of the bacterium to others. Penicillin is the recommended antibiotic with erythromycin as an alternative. Despite sporadic reports of antibiotic resistance to single antibiotics in *C.diphtheriae* (Harnisch *et al,* 1989), resistance has not been a particular problem. The antibiotic sensitivities of strains of *C.diphtheriae* isolated at this center between 1992 and 1996 has shown that 24% were resistant to tetracycline, 15% to erythromycin, 8% to chloramphenicol, 3% to trimethoprim and 1% to rifampicin (Parry *et al,* 1997b). Some strains exhibited resistance to two, three and four antibiotics. Although we have no evidence that the resistance was associated with a poorer clinical outcome, the emergence of multiple-antibiotic resistant diphtheria, not previously reported, is a worrying development.

MECHANISMS AND GENETICS OF ANTIBIOTIC RESISTANCE

Microorganisms have shown a remarkable ability to develop resistance to each successive generation of chemotherapeutic agents. The three main mechanisms of resistance to antimicrobial agents are shown in Table 1 (French and Phillips, 1997). Although some bacteria are inherently resistant to many commonly used antibiotics *(eg Pseudomonas aeruginosa, Burkholderia pseudomallei),* resistance more commonly emerges by the selection of resistance mutants or the acquisition of resistance genes.

The earliest forms of resistance arose due to point mutations in the gene coding for the target molecule of the antimicrobial agent. This is the case, for example, with *Mycobacterium tuberculosis* (Zhang and Young, 1994). Quinolone resistance in *S.typhi* also occurs by mutation. Analysis of strains from Vietnam have shown single base pair mutations at one of two sites in the quinolone resistance determining region of the *GyrA* gene (Wain *et al,* 1997). This mutation results in substitution of aspartate for glycine at position 87 or the substitution of serine for phenylalanine at position 83. Similar mutations have been found in quinolone resistant *S.typhi* from India (Brown *et al,* 1996). It has been recently recoginized that some bacteria mutate at a much higher rate than was previously thought. Strains of *E.coli* and *S.typhimurium* have been described that mutate at a rate one hundred times faster than previously thought possible (LeClerc *et al,* 1996). The mechanism of penicillin resistance in *S.pneumoniae* and *Neisseria gonorrhoea* is also by alteration of the target molecule. However in this case the altered penicillin binding protein (PBP) genes are believed to have arisen by

Table 1

Mechanisms of antibiotic resistance.

Mechanism	Example
Drug modifying enzymes	Beta lactamases (beta lactams, cephalosporins, carbapenems) Aminoglycoside-modifying enzymes Chloramphenicol acetyltransferase
Alteration of the target molecule	Penicillin binding proteins (penicillin, methicillin) DNA gyrase (fluoroquinolone) Ribosome (macrolide-licosamide) Dihydropteroate synthetase (sulphonamide) Dihydrofolate reductase (trimethoprim) Bacterial ligase (glycopeptide)
Alteration in permeability and drug efflux	Active efflux (tetracyclines) Decreases in porin proteins

interspecies homologous recombinational events (Spratt, 1994). It is thought that segments of the PBP genes have been replaced by sements from related species and resulted in mosaic genes.

Probably the most important mechanism contributing to the emegence and dissemination of resistance genes is the process of transferable resistance first described in *Shigella dysenteriae* (Watanabe and Fukasawa, 1960). Resistance genes may be carried on transferable genetic elements such as plasmids, transposons and integrons (Davies, 1994). Plasmids are extrachromosomal genetic elements that are autonomous and self-replicating. They may spread between bacteria of the same species and bacteria of different species by the processes of conjugation and transformation (Courvalin,1994). Plasmids can determine a wide variety of functions in bacteria such as metabolic capacities, virulence and antibiotic resistance. They can be classified by incompatibility grouping and restriction enzyme digestion. The chromosome or plasmid may have specialized genetic elements known as transposons. Transposons are small genetic elements that can move from one area of the chromosome to another or between the chromosome and plasmid and carry resistance genes. Mobile genetic elements such as plasmids and transposons enable antibiotic resistance genes to be disseminated within and between bacterial species. A single clone of a specific organism may become resistant when it inherits a plasmid or transposon. In the presence of suitable antibiotic pressure the resistant strain will be favored. The presence of several antimicrobial resistance genes on one element allows one antibiotic to select resistance to several unrelated antibiotics.

The antibiotic resistance plasmids in *S.typhi* are believed to have probably originated from plasmids in the Enterobacteriaceae commonly found in the gastrointestinal tract. Multiple-antibiotic resistance in *S. typhi* has generally been associated with large molecular weight incH1 plasmids (Mirza *et al*, 1996). Analysis of *S.typhi* isolated in Vietnam has shown that the multiple-antibiotic resistance genes are carried on large molecular weight self-transferable plasmids (unpublished observations).

Multiple-antibiotic resistant plasmids may evolve in a variety of ways. Site-specific integration of antibiotic resistant determinants may be mediated by a family of DNA elements called integrons. Integrons are small genetic units that function as gene expression cassettes. They may carry antimicrobial resistance genes and are able to insert into transposons, plasmids and chromosmes (Reechia and Hall, 1995). They have an integrase to allow site specitic integration and space for resistance genes to be inserted. An integral promotor

allows the resistance genes to be switched on and off and there are genes that appear to exchange freely between different integrons. The sequences of these integrons are common in drug resistant bacteria (Jones *et al*, 1997). Alternatively individual genes on the plasmid may also evolve by mutational events. Mutations in the original beta lactamase genes such as TEM1 and 2 and SHV1 have resulted in extended spectrum beta lactamase enzymes, active against the third generation cephalosporins and carbapenems (Davies, 1994).

SPREAD OF ANTIBIOTIC RESISTANCE

Antibiotic resistant bacteria may appear and spread in a new area by a variety of means as outlined in Table 2. Antibiotic usage is a key factor influencing the emergence of antibiotic resistance (McGowan, 1983). Antibiotic pressure will select for resistant mutants in a population and also allow strans with newly acquired resistance genes a selective advantage. This will particularly be the case where there is incorrect and unregulated antimicrobial usage. Antibiotic usage, however, is not the only factor. Person to person spread is also important, be it in hospitals, child-care facilities or overcrowded shanty towns. Furthermore, the increasing mobility and mixing of human populations is allowing the global spread of resistant bacteria.

Table 2

Factors conributing to the emergence of antibiotic resistant bacteria.

1. Acquisition of resistance in a previously susceptible strain (By conjugation, transformation, recombination).
2. Antibiotic selection of a sub-population of resistant mutants.
3. Person to person spread of a resistant clone.
4. Introduction of a resistant microorganism where none was previously present.

It is important to consider the bacterial ecosystem in which antimicrobial agents act. They do not merely exert evolutionary pressure on pathogens but also the normal bacterial flora of humans, animals and the environment. All humans have an enormous normal bacterial flora on the skin, in the oropharynx and gut. These bacterial flora are in a constant state of interchange with bacteria in animals, plants and the environment. Furthermore there is a constant and free exchange of genetic material between bacteria within and between these different ecosystems. When these large numbers of bacteria at these sites with a rapid reproductive rate are bathed in antibiotic, particularly at sub-therapeutic doses there is a potential for the rapid evolution of resistance. There is a circulating pool of resistance genes in the normal bacterial flora which are potentially transferred to pathogenic microorganisms.

This concept is well recognized in the hospital environment but is also important in the community as illustrated by the high levels of resistance that may be found in the normal enteric flora of healthy schoolchildren. Table 3 summarizes several studies in which antibiotic resistance to commonly used antibiotics were measured in *E.coli* isolated from the feces of healthy schoolchildren (Lester *et al*, 1990; Aymes *et al*, 1992; Mamun *et al*, 1993). In India there was a marked difference in the levels of resistance in children from rural communities compared to urban children, a difference also seen in the carriage of penicillin resistant pneumococci in Vietnamese children (Aymes *et al*, 1992; Parry *et al*, 1997a). This probably reflects different levels of antibiotic availability and usage and also of overcrowding.

Antibiotic usage is not only important in humans but also in the animal industry and agriculture (Piddock, 1996). In some countries the amounts of antibiotic used in these areas is equal to or more than that in humans. Increases in fluoroquinolones resistance in non typhi *Salmonellae* and *Campylobacter* and the emergence of vancomycin resistance in enterococci have been linked to the veterinary use of antibiotics.

Table 3

Antibiotic resistnce of fecal *E.coli* in healthy children.

Country	Reference	% resistance				
		Ampicillin	Trimethoprim	Chloramphenicol	Tetracycline	Sulphonamide
Bangladesh	Mamun *et al,* 1993	58-94	17-70	29-94	29-92	20-87
China	Lester *et al,* 1990	47	64	42	92	87
India: rural	Aymes *et al,* 1992	41	41	41		
India: urban	Aymes *et al,* 1992	94	95	94		
USA	Lester *et al,* 1990	23	3	15	33	36

WHAT CAN BE DONE?

Does this spread of infectious agents resistant to antimicrobial agents herald the start of the post-antibiotic era? And if so, whan can be done now to prevent the situation deteriorating? Possible steps include more efforts to prevent infection, careful surveillance of resistance and more careful use of existing antibiotics in addition to the development of new antibiotics.

Prevention of infection by the use of vaccines and improved public health strategies is clearly the best way of countering this problem but it will never be the whole answer. Surveillance of resistance to antimicrobials is important to define the extent and degree of the problem and as a prelude to reducing it. The provision of microbiological facilities and expertise for this in many areas of the world is indequate. Good surveillance is required on a local, national and international level. Molecular typing methods allow the possibility of tracking the national and international spread of resistant clones. A network of laboratories in each region, co-ordination by WHO and use of the internet could help to achieve this.

Control of antimicrobial usage that is the most difficult and controversial area. Before physicians are willing to change prescribing habits they must be convinced that such changes will make a difference and the evidence that it does is scanty. Furthermore much of the global antimicrobial usage is either over the counter in the private sector or veterinary use. What difference will a change in physician prescribing make if these other routes are unchecked?

One possibility is to reduce prescribing of a particular antibiotic or group of antibiotics. There are examples where changes of prescribing have resulted in a fall in levels of resistance. In an intensive care unit in Bahrain, for example, a major problem with *Klebsiella* resistance to the third generation cephalosporins developed following widespread use of these agents for treatment and prophylaxis (Wallace *et al,* 1995). A ban on the use of third generation cephalosporins and more careful use of other antibiotics resulted in a fall in the number of *Klebsiella* isolates resistant to third generation cephalosporins. The decline of chloramphenicol resistance in *S.typhi* in Thailand and Vietnam in the 1970s and 1980s as treatment changed to cotrimoxazole is another example (Butler *et al,* 1977; Hoa *et al,* 1992; Thisyakorn *et al,* 1987). Unfortunately this change was not permanent as MDR typhoid emerged in the 1990s. In Finland erythromycin resistance in *Streptococcus pyogenes* reached high levels the end of the 1980s. A countrywide effort to reduce macrolide consumption resulted in a significant fall in the level of erythromycin resistance (Seppala *et al,* 1997). In Hungary a reduction in penicillin usage was followed by a reduction in the incidence of penicillin resistant pneumococci (Nowak, 1994). Whether these reductions will be maintained remains to be seen.

More careful use of antimicrobial agents rather than a mere reduction in usage may also lead to a reduction in rates of resistance. An example of this is the introduction of directly oberved therapy for the treatment of tuberculosis. The change to directly observed therapy instead of traditional therapy (which relies on patient compliance) in Tarrant

County, Texas resulted in a significant falls in the level of drug resistance. Primary drug resistance in tuberculosis isolates fell from 13.0% to 6.7% ($p<0.001$), acquired resistance from 14.0% to 2.1% ($p<0.001$) and the relapse rate fell from 20.9% to 5.5% ($p<0.001$) (Weis *et al,* 1994). Treatment of tuberculosis with a single drug invariably fails due to the selection of resistant mutants. To reduce the chance of this occurring, multiple drugs with different resistant mechanisms are used simultaneously (Zhang and Young, 1994). No single bacterium is likely to be resistant to all the drugs used. The routine use of multiple drugs is a strategy now used for HIV and has recently been proposed for malaria (White, 1998). It is an approach that perhaps should be more widely used. There has been little research on the effect on resistance of innovative drug regimens such as rotating antibiotics or combinations.

Control of the usage of existing antibiotics is particularly difficult in the community. Many antimicrobial agents are bought over the counter in pharmacies or in the market rather than following the assessment and prescription of a physician. In study in Kenya 80% of antimalarials for febrile children were bought by mothers from the local pharmacy (Snow *et al,* 1992). Prescribing from pharmacies and doctors can be poor. In a study from Bangladesh only 8-10% of pharmacists and 43-46% of doctors knew the WHO recommended treatment for dysentery (Ronsmans *et al,* 1996). Adequate education and dissemination of information to those in the front line is critical.

We should not expect a host of new antibiotics to be available in the near future. It is a salutary lesson that until recently there have been no genuinely new antimicrobial agents developed in the last twenty years. Although some new agents are emerging such as the streptogramins, oxazolidones, everninomicins and antimicrobial peptides, they have a restricted market and are likely to be expensive (Mitchell, 1998). Increased understanding of the physiology, biochemistry and genetics of microorganisms allows the possibility of developing compounds to act against new therapeutic targets (Chopra *et al,* 1997). It is likely, however, that the ingenuity of bacteria will continue to circumvent old and new antimicrobial agents.

The emergence and dissemination of resistance to many antimicrobial agents threatens to reverse the benefit we have enjoyed in the last fifty years. To reduce the chance of the emergence of untreatable infections efforts must be made now to prevent infection, improve the surveillance of resistance, find better ways to use existing drugs as well as develop new agents. A co-ordinated and global approach is needed to prevent the spectre of untreatable infections in the 21st Century.

ACKNOWLEDGEMENTS

I gratefully acknowledge the help and support of the hospital leaders and the staff of the microbiology laboratory of the Center for Tropical Diseases, Ho Chi Minh City. I am also grateful for the invaluable help of Dr TT Hien, Dr TS Diep, Dr NTT Hoa, Mr John Wain, Dr JJ Farrar and Dr NJ White. The program of research at the Wellcome Trust Clinical Research Unit, Center for Tropical Diseases, Ho Chi Minh City is supported by the Wellcome Trust of Great Britain.

REFERENCES

Anand AC, Kataria VK, Singh W, Chatterjee SK. Epidemic multi-resistant enetric fever in eastern India. *Lancet* 1990; i: 352.

Aymes SGB, Tait S, Thomson CJ, *et al.* The incidence of antibiotic resistance in aerobic flora in South India. *J Antimicrob Chemother* 1992; 29: 415-25.

Brown JC, Shanahan PMA, Jesudason MV, Thomson CJ, Aymes SGB. Mutations responsible for reduced susceptibility to 4-quinolones in clinical isolates of multi-resistant *Salmonella typhi* in India. *J Antimicrob Chemother* 1996; 37: 891-900.

Butler T, Linh NM, Arnold K, Adickman MD, Chua DM, Muoi MM. Therapy of antimicrobial-resistant typhoid fever. *Antimicrob Agents Chemother* 1977; 21: 645-50.

Chopra I, Hodgson J, Metcalf B, Poste G. The search for antimicrobial agents effective against bacteria resistant to multiple antibiotics. *Antimicrob Agents Chemother* 1997; 41: 497-503.

Courvalin P. Transfer of antibiotic resistance genes between Gram-positive and Gram-negative bacteria. *Antimicrob Agents Chemother* 1994; 38: 1447-51.

Davies J. Inactivation of antibiotics and dissemination of resistance genes. *Science* 1994; 264: 375-82

French GL, Phillips I. In: O'Orady F, Lambert HP, Finch RG, Greenwood D, eds. Resistance in Antibiotic and Chemotherapy, 7th ed. 1997: 23-43.

Harnisch JP, Tronca E, Nolan CM, Turck M, Holmes KK. Diphtheria among alcoholic urban adults. A decade of experience in Seattle. *Ann Intern Med* 1989; 111: 71-82

Hien TT, Bethell DB, Hoa NTT, *et al.* Short course ofloxacin for treatment of multidrug-resistant typhoid. *Clin Infect Dis* 1995; 20: 917-23.

Hoa NTT, Phi LT, Hien TT, Smith MD, White NJ. Antibiotic resistance in *Salmonella typhi* and *S.paratyphi* A from Vietnam. [Abstract WeO9-6.] Thirteenth International Congress for Tropical Medicine and Malaria, Pattaya, Thailand. 1992.

Jesudason MV, Malathy B, John TJ. Trend of increasing levels of minimum inhibitory concentration of ciprofloxacin to *Salmonella typhi. Indian J Med Res* 1996; 103: 247-9.

Jones ME, Peters E, Weersink AM, Fluit A, Verhoef J. Widespread occurrence of integrons causing multiple antibiotic resistance in bacteria. *Lancet* 1997; 349: 1742-3.

LeClerc JE, Li B, Payne WL, Cebula TA. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* 1996; 274: 1208-11.

Lee HJ, Park JY, Jang SH, Kim JH, Kim EC, Choi KW. High incidence of resistance to multiple antimicrobials in clinical isolates of *Streptococcus pneumoniae* from a university hospital in Korea. *Clin Infect Dis* 1995: 20: 826-35.

Lester SC, Pilar Pla MD, Wang F, Schael IP, Jiang H, O' Brien TF. The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela and in Qin Pu, China. *N Engl J Med* 1990; 323: 285-9.

Mamun KZ, Shears P, Hart CA. The prevalence and genetics of resistance to commonly used antimicrobial agents in faecal Enterobacteriaceae from children in Bangladesh. *Epidemiol Infect* 1993; 110: 447-58.

McGowan JE. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. *Rev Infect Dis* 1983; 5: 1033-48.

Mitchell P. Rearming in the fight against bacteria. *Lancet* 1998; 352: 462.

Mirza SH, Beeching NJ, Hart CA. Multi-drug resistant typhoid: a global problem. *J Med Microbiol* 1996; 44: 317-9.

Neu HC. The crisis in antibiotic resistance. *Science* 1992; 257: 1064-73.

Nowak R. Hungary sees an improvement in penicillin resistance. *Science* 1994; 264: 364.

Olarte J, Galindo E. *Salmonella typhi* resistant to chloramphenicol, ampicillin, and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. *Antimicrob Agents Chemother* 1973; 4: 597-601.

Parry CM, Diep TS, Hoa NTT, *et al.* Penicillin resistance in *Streptococcus pneumoniae* isolated from adults and children in and around Ho Chi Minh City, Vietnam. Joint International Tropical Medicine Meeting, Bangkok, Thailand. 25-27 August 1997a: 63.

Parry CM, Hoa NTT, Wain J, *et al.* The *in vitro* susceptibilities of *Corynebacterium diphtheriae* isolated between 1992 and 1996 in Vietnam [Abstract]. 35th Annual meeting of the Infectious Diseases Society of America. San Fransisco. 13-16 September 1997b.

Parry C, Wain J, Chinh NT, Vinh H, Farrar JJ. Quinolone-resistant *Salmonella typhi* in Vietnam. *Lancet* 1998; 351: 1289.

Piddock LJV. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy? *J Antimicrobial Chemother* 1996; 38: 1-3.

Recchia GD, Hall RM. Gene Cassettes: a new class of mobile element. *Microbiology* 1995: 141: 3015-27.

Ronsmans C, Islam T, Bennish ML. Medical practitioners knowledge of dysentery treatment in Bangladesh. *Br Med J* 1996; 313: 205-6.

Seppala H, Klaukka T, Vuopo-Varkila J, *et al.* The Finnish Study Group for Antimicrobial Resistance. *N Engl J Med* 1997; 337: 441-6.

Shears P. A review of bacterial resistance to antimicrobial agents in tropical countries. *Ann Trop Paediatr* 1993; 13: 219-6.

Snow RW, Peshu N, Forster D, Mwenesi H, Marsh K. The role of shops in the treatment and prevention of childhood malaria on the coast of Kenya. *Trans R Soc Trop Med Hyg* 1992; 86: 237-9.

Spratt B. Resistance to antibiotics mediated by target alterations. *Science* 1994: 264: 388-93.

Sturm AW, van der Pol R, Smits AJ, *et al.* Over-the-counter availability of antimicrobial agents, self medication and patterns of resistance in Karachi, Pakistan. *J Antimicrob Chemother* 1997: 39: 543-7.

Thisyakorn U, Mansuwan P, Taylor DN. Typhoid fever and paratyphoid fever in 192 hospitalised children in Thailand. *Am J Dis Child* 1987; 141: 862-5.

Tomasz A. Multiple-antibiotic-resistant pathogenic bacteria. *N Engl J Med* 1994; 330: 1247-51.

Wain J, Hoa NTT, Chinh NT, *et al.* Quinolone-resistant *Salmonella typhi* in Viet Nam: Molecular basis of resistance and clinical response to treatment. *Clin Infect Dis* 1997; 25: 1404-10.

Wallace MR, Johnson AP, Daniel M, Malde M, Yousif AA. Sequential emergence of multiresistant *Klebsiella pneumoniae* in Bahrain. *J Hosp Infect* 1995; 31: 247-22.

Watanabe T, Fukasawa T. Episome mediated transfer of drug resisance in Enterobacteriaceae. Transfer of resistance factors by conjugation. *J Bacteriol* 1960; 81: 669-77,

Weis SE, Slocum PC, Blais FX, *et al.* The effect of directly observed therapy on the rates of drug resistance and relapse in tuberculosis. *N Engl J Med* 1994; 330: 1179-84.

White NJ, Parry CM. The treatment of typhoid fever. *Curr Opin Infect Dis* 1996; 9: 298-302.

White NJ. Preventing antimalarial drug resistance through combinations. *Drug Resistance Updates* 1998; 1: 3-9.

Woodward TE, Smadel JE, Ley HL, Green R, Mankikar DS. Preliminary report on the beneficial effect of cholormycetin in the treatment of typhoid fever. *Ann Intern Med* 1948; 29: 131-34.

Yoshida R, Kaku M, Kohno S, *et al.* Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *Antimicrob Agents Chemother* 1995; 39: 1196-8.

Young H-K. Antimicrobial resistance spread in aquatic environments. *J Antimicrob Chemother* 1993; 31: 627-35.

Zhang Y, Young D. Molecular genetics of drug resistance in *Mycobacterium tuberculosis. J Antimicrob Chemother* 1994; 34: 313-9.