Upsala Journal of Medical Sciences. 2014; 119: 142–148

informa
healthcare

REVIEW ARTICLE

Reversibility of antibiotic resistance

MARTIN SUNDQVIST

Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden

Abstract
Although theoretically attractive, the reversibility of resistance has proven difficult in practice, even though antibiotic resistance mechanisms induce a fitness cost to the bacterium. Associated resistance to other antibiotics and compensatory mutations seem to ameliorate the effect of antibiotic interventions in the community. In this paper the current understanding of the concepts of reversibility of antibiotic resistance and the interventions performed in hospitals and in the community are reviewed.

Key words: *Antibiotic resistance, fitness, intervention*

Introduction

Bacteria can acquire antibiotic resistance through mutations (1), or through incorporation of DNA from other bacteria via horizontal gene transfer (2). Long before the introduction of antibiotics for treatment of infections, environmental bacteria had developed various antibiotic resistance mechanisms in response to the presence of antibacterial substances produced by other organisms in their environment or by themselves (3). In clinical isolates from the pre-antibiotic era plasmids able to transfer genetic elements by conjugation were present (4,5), but antibiotic resistance was not expressed (3). The introduction of resistance mechanisms in these plasmids, the development of new resistance mechanisms, and the spread of these mechanisms thus seem to have occurred during the last 70 years (3).

A bacterium will acquire antibiotic resistance as a response to the environment. Although *mutations* will cause antibiotic resistance in some cases (1), the most important mechanism for the acquisition of antibiotic resistance is *horizontal gene transfer* (HGT), in Enterobacteriaceae mainly mediated by conjugation (2). The acquisition of the mutation/resistance gene might impose a *fitness cost*, usually measured as a decreased growth rate, on the bacterium (6–12). The fitness cost will be deleterious to the newly resistant strain unless exposed to a continuous antibiotic *selection* (9) and/or a rapid development of compensatory mutations (13,14). The antibiotic selection pressure will additionally contribute to the dissemination of the resistance gene both through clonal expansion and through spread to new bacteria of the same or other species (15). The clonal expansion within the host will increase the probability of further successful *spread* to other hosts (15). For the 'newly born' resistance mechanism to become clinically significant, the mechanism should affect a clinically used antibiotic and be introduced into a pathogen with high epidemic potential (16–18). The newly incorporated resistance mechanism is unlikely to be the only resistance mechanism in the bacterium as an isolate resistant to one antibiotic is prone to be resistant to one or more other antibiotics. This phenomenon is called *associated resistance* (19). The observed fitness cost of resistance genes/mutations (6–12) is a prerequisite for *reversibility* of antibiotic resistance by reduced antibiotic use. Mathematical models have predicted a faster reversibility in hospital settings than in the community

Correspondence: Martin Sundqvist, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, 70185 Örebro, Sweden.
E-mail: martin.sundqvist@orebroll.se

(Received 9 January 2014; accepted 5 March 2014)

ISSN 0300-9734 print/ISSN 2000–1967 online
DOI: 10.3109/03009734.2014.903323

due to the dynamics of individuals moving in and out of the system studied (20). However, so far the clinical evidence for reversibility is limited (21,22).

It has been shown that some antibiotic resistance mechanisms confer a measurable fitness cost *in vitro* (9,12,23). The postulated fitness cost has influenced the construction of mathematical models on antibiotic resistance development (20,24,25), leading to the idea that a reduction in antibiotic use would counteract the selection of resistant organisms and allow expansion of more fit susceptible strains and/or loss of genes encoding antibiotic resistance. This would result in a drop in the frequency of resistance as measured in bacteria isolated in clinical samples. These *in vitro* studies have typically measured the fitness cost as the difference in growth rate between strains with and without resistance to defined agents (9,12,23). Experimentally, resistance has been incorporated in well-defined susceptible strains through HGT (26) or by induction of resistance mutations (9). The constructed iso-genic pair is allowed to compete in a common culture. The strain dominating the culture after a certain number of generations is considered the more fit strain. Only occasionally have horizontally transferred resistance mechanisms been shown to confer a substantial fitness cost (27). Sometimes such acquisition of resistance has been neutral (26–28) or even beneficial for the studied strain (29–33).

However, measuring growth rate is not the only way of estimating fitness. Several other factors will help to determine the survival and establishment of resistant strains in bacterial populations. For example, the incorporation of an *Escherichia coli* strain in the intestinal flora will depend on the growth rate but also on features affecting colonization and virulence of the resistant strain (i.e. adherence factors etc.) (34). Additionally the flora composition at challenge (35) and the selective pressure by antibiotics (36) will affect the survival of the strain. All these factors taken together will affect the epidemiological fitness (24,37–39) of a resistance mechanism—growth rate, transmission capacity (39), transmission dynamics in relation to selection (37), and persistence despite a lack of selective pressure (38). In this context there is a special interest in uncommon antibiotic resistance. In *E. coli*, resistance to both mecillinam and nitrofurantoin is typically reported to be below 5%. We have shown that these isolates are distributed within the whole *E. coli* population and do not seem to aggregate in successful uropathogenic clones (40). This might be due to the fact that both nitrofurantoin resistance (12) and mecillinam resistance (unpublished data) are associated with a fitness cost. In this case the fitness cost seems to prohibit these antibiotic resistance mechanisms to become epidemiologically successful.

Interventions on antibiotic use

In agriculture the ban on avoparcin caused an initial dramatic decrease in the levels of glycopeptide resistance in enterococci isolated from pigs and pig farm environments. This was facilitated by the normal fate of most pigs, but, interestingly, low levels of glycopeptide resistance still remain on the investigated farms and is predicted to remain for a long time (41). The use of antibiotics changes over time. Only rarely do we manage to design drugs with novel targets in bacteria. There are instances where resistance development has necessitated drastic changes in antibiotic use. In other cases the discovery of serious side effects has caused pronounced shifts. Planned interventions on antibiotic use to reduce antibiotic resistance have been performed mainly in hospital settings, but there are a few examples of interventions in communities, usually in response to emerging resistance problems.

Hospital

A number of shifts in antibiotic policy in hospitals in response to the emergence of resistance problems have been reported over the years (42–48). Most of these studies have been in response to a situation where a ward or a hospital has experienced a high level of resistance to third-generation cephalosporins or carbapenems. In some of these the high levels have been due to a high prevalence of a specific resistant clone (46). Some have shown a decisive reduction in antibiotic resistance in response to an antibiotic shift (45,47), while others have reported no effects (43). In several of these studies there has been an increase in resistance to other antibiotics, carbapenems (47) and fluoroquinolones (48). The overall effect on antibiotic resistance of interventions focusing on a single antibiotic in hospitals can thus be questioned. However, some programmes with antibiotic cycling strategies in wards highly exposed to a single broad-spectrum antibiotic have been proven effective to reduce the rate of bacteria producing extended spectrum beta-lactamases (ESBL) (49). Although these approaches have been reported successful, the trend in almost all reports from all over the world shows increasing rates of antibiotic-resistant bacteria. Thus, patients will increasingly often be colonized with multi-resistant bacteria already at admission (50–53), and the effect of a sudden shift in antibiotic policy to reduce the spread of these organisms in hospitals will eventually diminish. Increasing multi-resistance will render any antibiotic an efficient selective force for keeping almost any resistance (48).

Community

In the community two large studies have shown a possibility of decreasing antibiotic resistance through community-wide interventions (21,22). Both studies were performed in response to a sudden dramatic increase in antibiotic resistance. The first study was conducted in Finland in response to a nationwide increase in erythromycin resistance in *Streptococcus pyogenes*. National recommendations advocating a decreased use of macrolide antibiotics were launched at the end of 1991 (21). During 1992 the use of macrolides decreased from 2.40 to 1.38 DDD/TIND and remained at this level during the study period. The increase in erythromycin resistance in *S. pyogenes* seen in 1992–1993 was followed by a significant decrease in 1994 and 1995. However, closer examination of the data presented in the article reveals that the use of macrolides significantly decreased already in 1989. The reduction in macrolide resistance was thus seen 5 years after the initial decrease in macrolide sales, indicating that other factors than the decreased use might have contributed to the decrease in resistance seen in 1994–1995. Later it was shown that one clone of *S. pyogenes*, being resistant to erythromycin only, seemed to be responsible for the increase in macrolide resistance (54). Thus, the observed differences might have been natural fluctuations, although probably enhanced both by the macrolide use in the late 1980s and later the decrease in macrolide consumption.

In Iceland, resistance to penicillin in *Streptococcus pneumoniae* increased from 1989 to 1993. This initiated a nationwide campaign to reduce the prescriptions of antibiotics. The reductions and changes in consumption together with restrictions on day care centre attendance for children colonized with non-susceptible *S. pneumoniae* resulted in a decrease in the frequency of penicillin non-susceptible pneumococci (PNSP) (22). However, in 2006 it was shown that this decrease was short-lived as a continued conservative use of antibiotics was not accompanied by a sustained low level of PNSP. This was attributed to a clonal spread of successful PNSP serotypes (55,56). Two recent studies on *S. pyogenes* (57) and *S. pneumoniae* (58) further emphasize the importance of successful resistant clones as an important cause of both the rise and fall of resistance in bacteria not considered as normal flora. The Korean study on *S. pyogenes* showed that a nationwide decrease in erythromycin resistance was due to a decrease of a certain resistant *emm*-type rather than due to decreased erythromycin use (57). In *S. pneumoniae*, successful multi-resistant clones have been shown to have increased fitness despite the accumulation of antibiotic resistance, challenging reduced antibiotic use to be effective in controlling these clones. A large proportion of these clones, however, belonged to serotypes covered in the 7-valent conjugate vaccine now being used, thus limiting their spread (58). In Enterobacteriaceae the impact of antibiotic restriction policies seems very limited. In 2010 we published a study on the possibility to reverse trimethoprim resistance in *E. coli* by decreasing trimethoprim use in the community during 2 years. The use of trimethoprim dropped dramatically by 85% at the start of the intervention and kept at this low level during 2 years. Despite this, the effect on trimethoprim resistance in *E. coli* was shown to be marginal, and the fraction of trimethoprim mono-resistant *E. coli* was not affected by the intervention. Clinical isolates of *E. coli* resistant to trimethoprim only or in combination with ampicillin and/or nalidixic acid resistance did not display any fitness cost *in vitro* (59). This finding might be due to compensatory mutations (60,61). The no-effect of the intervention was judged to be due to the non-existing fitness cost and the high levels of associated resistance to other antibiotics used during the intervention (59). Generalizing from the results from this study, reducing the consumption of one antibiotic might delay the development of antibiotic resistance but not reverse it. This is in line with the earlier, retrospective, studies on *E. coli* from Great Britain where trimethoprim-sulphamethoxazole was used until 1991 when its popularity gradually declined because of its adverse effects. Formal prescribing restrictions were implemented in 1995, and in the period up to 1999 there was a 97% decrease in the consumption of trimethoprim-sulphamethoxazole. Despite this, the frequency of resistance to sulphamethoxazole among *E. coli* was virtually unchanged in 2004 (62,63). Partly using the same set of isolates, it was later shown that streptomycin resistance remained stable although the use in Great Britain had been very low for the last 30 years (63,64). In this perspective it is important to highlight that after the intervention in Kronoberg county trimethoprim resistance has increased dramatically from 11%–13% in 2008 to a level around 19% in *E. coli* in 2013 (personal communication G. Kahlmeter).

Two more recent studies have, however, shown that a reduction in antibiotic use could result in decreased resistance frequencies in *E. coli* (65,66). Butler and co-workers followed the antibiotic prescriptions and resistance rates in a large number of Primary Health care centers (PHCCs) in Wales and could show that the PHCCs having the largest reduction in ampicillin (AMP) and trimethoprim (TMP) prescriptions during 7 years also experienced a decrease in the corresponding resistance in *E. coli* in their communities. The decrease was 58.7% to 53.5% for AMP and 29.1% to 25.7% for TMP resistance (65). No data were presented giving the reader a chance to evaluate whether

Table I. Studies evaluating the effect on resistance rates of large-scale reductions in antibiotic use in the community.

Country (ref.)	Species	Antibiotic(s)	Intervention/evaluation	Study design	Resistance frequency
Finland (21)	*S. pyogenes*	Macrolides	Nationwide/nationwide	Prospective	Decrease
Island (22)	*S. pneumoniae*	β-lactams and more	Nationwide/nationwide	Prospective	Decrease
Great Britain (62,63)	*E. coli*	SXT	Nationwide/local	Retrospective	Increase
Great Britain (64)	*E. coli*	streptomycin	Nationwide/local	Retrospective	No effect
Sweden (59)	*E. coli*	TMP, SXT	County/county	Prospective	Marginal effect
Great Britain (65)	*E. coli*	AMP, TMP, and more	PHC/PHC	Retrospective	Decrease
Israel (66)	*E. coli*	FQX	County/county	Retrospective	Decrease

AMP = Ampicillin; FQX = Fluoroquinolones; SXT = Trimethoprim-sulfamethoxazole; TMP = Trimethoprim.

the small decrease was continuous or extrapolated from two measurements. Importantly, the decrease was from a very high level of resistance, and the change could not possibly affect the empirical utility of these drugs. Gottesman and co-workers presented data on ciprofloxacin resistance in *E. coli* in relation to a nationwide restriction in ciprofloxacin use in Israel (due to a threat of anthrax attacks). Interrupted time series analysis showed a significant decrease in ciprofloxacin resistance from 12% to 9% in the course of a 6-month intervention, gradually reaching a reduction from 7,000 DDD/month to 4,500 DDD/month (66). The study has several limitations. There was little information on methodological or interpretative aspects of susceptibility testing. Thus, associated resistance rates in fluoroquinolone-resistant *E. coli* were not reported or discussed. The rapid effect on resistance rates is theoretically unlikely as recent data indicate the initial fitness cost of fluoroquinolone resistance in *E. coli* to be rapidly compensated for (14).

In view of the results of the seven studies performed so far on the effects of reduced antibiotic use in the community (Table I), together with the pronounced associated resistance rates and seemingly low epidemiological fitness cost of resistance determinants, we should probably not expect that even major antibiotic interventions will dramatically affect resistance rates in the community (41). However, new strategies might prove efficient to counteract resistance development in some species. Vaccination against important serotypes of *S. pneumoniae* has been proven effective in reducing antibiotic resistance in clinical infections (58,67).

Conclusions

The potential of reversing antibiotic resistance through the reduction of antibiotic use will be dependent on the fitness cost of the resistance mechanism, the epidemic potential of the bacteria/strain, and the transmission route of the species. Both *S. pyogenes* and *S. pneumoniae* have a well-known epidemic potential (68–72). The transmission routes for *E. coli* and other Enterobacteriaceae are more indirect and will be dependent on living standards, water, and food supply (73,74). These differences are probably of great importance and should be incorporated in new strategies aiming at limiting the burden of antibiotic resistance.

So far, we may have overestimated the usefulness of a strategy for reversing antimicrobial resistance based on the fitness cost of resistance. We have at the same time underestimated the conserving effects of associated resistance and the fundamental importance of clonal spread of both susceptible and resistant clones especially among bacteria not being part of our commensal flora. We can expect every molecule of antibiotics wherever present to select for the persistence of antibiotic-resistant strains (75). The normal faecal flora is an important reservoir for the development and selection of resistant bacteria. The carriage of ESBL-producing *E. coli* has increased dramatically during the last years, underlining the importance of the continuous selection of subpopulations of already resistant bacteria in the faecal flora. Globalization, the rapid and frequent traveling and the increasing international market exchange of foods and feeds, and modern health care will increase the spread and selection of resistant bacteria favouring the persistence of multi-resistant bacteria.

Importantly, this situation, where antibiotic resistance does not seem obviously reversible, must not make us reluctant to impose the measures that might postpone the increase in antibiotic resistance. The overall use of antibiotics must be reduced. The prudent use of antibiotics should always be promoted. Prudent means: always appropriate, less in many cases, but occasionally some patients will need

more and broader treatment. Narrow-spectrum antibiotics with low impact on the normal flora should in most cases be promoted, and antibiotics with a low propensity of clonal spread of resistance due to high fitness costs should be used. In addition vaccination strategies should be implemented where appropriate both to reduce the burden of antibiotic resistance but also to reduce the need for antibiotic treatment, and stringent enforcement of infection control measures within hospitals must be instituted and rigorously upheld in every institution involved in health care.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Martinez JL, Baquero F. Mutation frequencies and antibiotic resistance. Antimicrob Agents Chemother. 2000;44:1771–7.
2. Barlow M. What antimicrobial resistance has taught us about horizontal gene transfer. Methods Mol Biol. 2009;532:397–411.
3. Hawkey PM. Molecular epidemiology of clinically significant antibiotic resistance genes. Br J Pharmacol. 2008;153:S406–13.
4. Hughes VM, Datta N. Conjugative plasmids in bacteria of the 'pre-antibiotic' era. Nature. 1983;302:725–6.
5. Datta N, Hughes VM. Plasmids of the same Inc groups in Enterobacteria before and after the medical use of antibiotics. Nature. 1983;306:616–17.
6. Nagaev I, Bjorkman J, Andersson DI, Hughes D. Biological cost and compensatory evolution in fusidic acid-resistant Staphylococcus aureus. Mol Microbiol. 2001;40:433–9.
7. Sander P, Springer B, Prammananan T, Sturmfels A, Kappler M, Pletschette M, et al. Fitness cost of chromosomal drug resistance-conferring mutations. Antimicrob Agents Chemother. 2002;46:1204–11.
8. Gustafsson I, Cars O, Andersson DI. Fitness of antibiotic resistant Staphylococcus epidermidis assessed by competition on the skin of human volunteers. J Antimicrob Chemother. 2003;52:258–63.
9. Komp Lindgren P, Marcusson LL, Sandvang D, Frimodt-Moller N, Hughes D. Biological cost of single and multiple norfloxacin resistance mutations in Escherichia coli implicated in urinary tract infections. Antimicrob Agents Chemother. 2005;49:2343–51.
10. Gagneux S, Long CD, Small PM, Van T, Schoolnik GK, Bohannan BJ. The competitive cost of antibiotic resistance in Mycobacterium tuberculosis. Science. 2006;312:1944–6.
11. Nilsson AI, Zorzet A, Kanth A, Dahlstrom S, Berg OG, Andersson DI. Reducing the fitness cost of antibiotic resistance by amplification of initiator tRNA genes. Proc Natl Acad Sci USA. 2006;103:6976–81.
12. Sandegren L, Lindqvist A, Kahlmeter G, Andersson DI. Nitrofurantoin resistance mechanism and fitness cost in Escherichia coli. J Antimicrob Chemother. 2008;62:495–503.
13. Schrag SJ, Perrot V, Levin BR. Adaptation to the fitness costs of antibiotic resistance in Escherichia coli. Proc Biol Sci. 1997; 264:1287–91.
14. Marcusson LL, Frimodt-Moller N, Hughes D. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. PLoS Pathog. 2009;5:e1000541.
15. Martinez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev. 2002;15:647–79.
16. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, et al. Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;61:273–81.
17. Johnson JR, Murray AC, Kuskowski MA, Schubert S, Prère MF, Picard B, et al. Distribution and characteristics of Escherichia coli clonal group A. Emerg Infect Dis. 2005;11:141–5.
18. Osterlund A, Eden T, Olsson-Liljequist B, Haeggman S, Kahlmeter G. Clonal spread among Swedish children of a Staphylococcus aureus strain resistant to fusidic acid. Scand J Infect Dis. 2002;34:729–34.
19. Kahlmeter G, Menday P. Cross-resistance and associated resistance in 2478 Escherichia coli isolates from the Pan-European ECO.SENS Project surveying the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections. J Antimicrob Chemother. 2003;52:128–31.
20. Lipsitch M, Levin BR. The population dynamics of antimicrobial chemotherapy. Antimicrob Agents Chemother. 1997; 41:363–73.
21. Seppala H, Klaukka T, Vuopio-Varkila J, Muotiala A, Helenius H, Lager K, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. N Engl J Med. 1997;337:441–6.
22. Kristinsson KG. Effect of antimicrobial use and other risk factors on antimicrobial resistance in pneumococci. Microb Drug Resist. 1997;3:117–23.
23. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, Andersson DI. Biological costs and mechanisms of fosfomycin resistance in Escherichia coli. Antimicrob Agents Chemother. 2003;47:2850–8.
24. Lipsitch M, Bergstrom CT, Levin BR. The epidemiology of antibiotic resistance in hospitals: paradoxes and prescriptions. Proc Natl Acad Sci USA. 2000;97:1938–43.
25. Levin BR, Lipsitch M, Perrot V, Schrag S, Antia R, Simonsen L, et al. The population genetics of antibiotic resistance. Clin Infect Dis. 1997;24:S9–16.
26. Enne VI, Delsol AA, Davis GR, Hayward SL, Roe JM, Bennett PM. Assessment of the fitness impacts on Escherichia coli of acquisition of antibiotic resistance genes encoded by different types of genetic element. J Antimicrob Chemother. 2005;56:544–51.
27. Marciano DC, Karkouti OY, Palzkill T. A fitness cost associated with the antibiotic resistance enzyme SME-1 beta-lactamase. Genetics. 2007;176:2381–92.
28. Johnsen PJ, Simonsen GS, Olsvik O, Midtvedt T, Sundsfjord A. Stability, persistence, and evolution of plasmid-encoded vanA glycopeptide resistance in Enterococci in the absence of antibiotic selection in vitro and in gnotobiotic mice. Microb Drug Resist. 2002;8:161–70.
29. Enne VI, Bennett PM, Livermore DM, Hall LM. Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. J Antimicrob Chemother. 2004; 53:958–63.
30. Yates CM, Shaw DJ, Roe AJ, Woolhouse ME, Amyes SG. Enhancement of bacterial competitive fitness by apramycin resistance plasmids from non-pathogenic Escherichia coli. Biol Lett. 2006;2:463–5.
31. Dahlberg C, Chao L. Amelioration of the cost of conjugative plasmid carriage in Eschericha coli K12. Genetics. 2003;165: 1641–9.

32. Mroczkowska JE, Barlow M. Fitness trade-offs in blaTEM evolution. Antimicrob Agents Chemother. 2008;52:2340–5.
33. Bean DC, Livermore DM, Hall LM. Plasmids imparting sulfonamide resistance in Escherichia coli: implications for persistence. Antimicrob Agents Chemother. 2009;53:1088–93.
34. Karami N, Nowrouzian F, Adlerberth I, Wold AE. Tetracycline resistance in Escherichia coli and persistence in the infantile colonic microbiota. Antimicrob Agents Chemother. 2006;50:156–61.
35. Sears HJ, Janes H, Saloum R, Brownlee I, Lamoreaux LF. Persistence of individual strains of Escherichia coli in man and dog under varying conditions. J Bacteriol. 1956;71:370–2.
36. Tacconelli E, De Angelis G, Cataldo MA, Mantengoli E, Spanu T, Pan A, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. Antimicrob Agents Chemother. 2009;53:4264–9.
37. Perron GG, Gonzalez A, Buckling A. Source-sink dynamics shape the evolution of antibiotic resistance and its pleiotropic fitness cost. Proc Biol Sci. 2007;274:2351–6.
38. Babiker HA. Seasonal fluctuation of drug-resistant malaria parasites: a sign of fitness cost. Trends Parasitol. 2009;25: 351–2.
39. Luciani F, Sisson SA, Jiang H, Francis AR, Tanaka MM. The epidemiological fitness cost of drug resistance in Mycobacterium tuberculosis. Proc Natl Acad Sci USA. 2009;106: 14711–15.
40. Poulsen HO, Johansson A, Granholm S, Kahlmeter G, Sundqvist M. High genetic diversity of nitrofurantoin- or mecillinam-resistant Escherichia coli indicates low propensity for clonal spread. J Antimicrob Chemother. 2013;68:1974–7.
41. Johnsen PJ, Townsend JP, Bohn T, Simonsen GS, Sundsfjord A, Nielsen KM. Factors affecting the reversal of antimicrobial-drug resistance. Lancet Infect Dis. 2009;9: 357–64.
42. Lepper PM, Grusa E, Reichl H, Hogel J, Trautmann M. Consumption of imipenem correlates with beta-lactam resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother. 2002;46:2920–5.
43. Cook PP, Catrou PG, Christie JD, Young PD, Polk RE. Reduction in broad-spectrum antimicrobial use associated with no improvement in hospital antibiogram. J Antimicrob Chemother. 2004;53:853–9.
44. Carling P, Fung T, Killion A, Terrin N, Barza M. Favorable impact of a multidisciplinary antibiotic management program conducted during 7 years. Infect Control Hosp Epidemiol. 2003;24:699–706.
45. Lee J, Pai H, Kim YK, Kim NH, Eun BW, Kang HJ, et al. Control of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in a children's hospital by changing antimicrobial agent usage policy. J Antimicrob Chemother. 2007;60:629–37.
46. Tängdén T, Eriksson B-M, Melhus Å, Svennblad B, Cars O. Radical reduction of cephalosporin use at a tertiary hospital after educational antibiotic intervention during an outbreak of extended-spectrum β-lactamase-producing Klebsiella pneumonia. J Antimicrob Chemother. 2011;66:1161–7.
47. Rahal JJ, Urban C, Horn D, Freeman K, Segal-Maurer S, Maurer J, et al. Class restriction of cephalosporin use to control total cephalosporin resistance in nosocomial Klebsiella. JAMA. 1998;280:1233–7.
48. Nijssen S, Fluit A, van de Vijver D, Top J, Willems R, Bonten MJM. Effects of reducing beta-lactam antibiotic pressure on intestinal colonization of antibiotic-resistant gram-negative bacteria. Intensive Care Med. 2011;36:512–19.
49. Chong Y, Shimoda S, Yakushiji H, Ito Y, Miyamoto T, Kamimura T, et al. Antibiotic rotation for febrile neutropenic patients with hematological malignancies: clinical significance of antibiotic heterogeneity. PLoS One. 2013;8:e54190.
50. Ortega M, Marco F, Soriano A, Almela M, Martínez JA, Muñoz A, et al. Analysis of 4758 Escherichia coli bacteraemia episodes: predictive factors for isolation of an antibiotic-resistant strain and their impact on the outcome. J Antimicrob Chemother. 2009;63:568–74.
51. Valverde A, Coque TM, Sanchez-Moreno MP, Rollan A, Baquero F, Canton R. Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae during nonoutbreak situations in Spain. J Clin Microbiol. 2004;42:4769–75.
52. Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, et al. High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol. 2008;46:2796–9.
53. Tacconelli E. New strategies to identify patients harbouring antibiotic-resistant bacteria at hospital admission. Clin Microbiol Infect. 2006;12:102–9.
54. Kataja J, Huovinen P, Muotiala A, Vuopio-Varkila J, Efstratiou A, Hallas G, et al. Clonal spread of group A streptococcus with the new type of erythromycin resistance. Finnish Study Group for Antimicrobial Resistance. J Infect Dis. 1998;177:786–9.
55. Arason VA, Sigurdsson JA, Erlendsdottir H, Gudmundsson S, Kristinsson KG. The role of antimicrobial use in the epidemiology of resistant pneumococci: a 10-year follow up. Microb Drug Resist. 2006;12:169–76.
56. Arason VA, Gunnlaugsson A, Sigurdsson JA, Erlendsdottir H, Gudmundsson S, Kristinsson KG. Clonal spread of resistant pneumococci despite diminished antimicrobial use. Microb Drug Resist. 2002;8:187–92.
57. Koh E, Kim S. Decline in erythromycin resistance in group A Streptococci from acute pharyngitis due to changes in the emm Genotypes rather than restriction of antibiotic use. Korean J Lab Med. 2010;30:485–90.
58. Rudolf D, Michaylov N, van der Linden M, Hoy L, Klugman KP, Welte T, et al. International Pneumococcal clones match or exceed the fitness of other strains despite the accumulation of antibiotic resistance. Antimicrob Agents Chemother. 2011;55:4915–17.
59. Sundqvist M, Geli P, Andersson DI, Sjölund-Karlsson M, Runehagen A, Cars H, et al. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. J Antimicrob Chemother. 2010;65:350–60.
60. Levin BR, Perrot V, Walker N. Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. Genetics. 2000;154:985–97.
61. Bjorkman J, Nagaev I, Berg OG, Hughes D, Andersson DI. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. Science. 2000;287:1479–82.
62. Enne VI, Livermore DM, Stephens P, Hall LM. Persistence of sulphonamide resistance in Escherichia coli in the UK despite national prescribing restriction. Lancet. 2001;357:1325–8.
63. Bean DC, Livermore DM, Papa I, Hall LM. Resistance among Escherichia coli to sulphonamides and other antimicrobials now little used in man. J Antimicrob Chemother. 2005;56:962–4.
64. Chiew YF, Yeo SF, Hall LM, Livermore DM. Can susceptibility to an antimicrobial be restored by halting its use? The case of streptomycin versus Enterobacteriaceae. J Antimicrob Chemother. 1998;41:247–51.

65. Butler CC, Dunstan F, Heginbothom M, Mason B, Roberts Z, Hillier S, et al. Containing antibiotic resistance: decreased antibiotic-resistant coliform urinary tract infections with reduction in antibiotic prescribing by general practices. Br J Gen Pract. 2007;57:785–92.
66. Gottesman BS, Carmeli Y, Shitrit P, Chowers M. Impact of quinolone restriction on resistance patterns of Escherichia coli isolated from urine by culture in a community setting. Clin Infect Dis. 2009;49:869–75.
67. Imöhl M, Reinert RR, Mutscher C, van der Linden M. Macrolide susceptibility and serotype specific macrolide resistance of invasive isolates of Streptococcus pneumoniae in Germany from 1992 to 2008. BMC Microbiol. 2010;10: 299.
68. Hausdorff WP, Feikin DR, Klugman KP. Epidemiological differences among pneumococcal serotypes. Lancet Infect Dis. 2005;5:83–93.
69. Sierra JMMDP, Sanchez FMDP, Castro PMD, Salvadó M, de la Red G, Libois A, et al. Group A Streptococcal infections in injection drug users in Barcelona, Spain: epidemiologic, clinical, and microbiologic analysis of 3 clusters of cases from 2000 to 2003. Medicine (Baltimore). 2006;85:139–46.
70. Crum NF, Barrozo CP, Chapman FA, Ryan MAK, Russell KL. An outbreak of conjunctivitis due to a novel unencapsulated Streptococcus pneumoniae among military trainees. Clin Infect Dis. 2004;39:1148–54.
71. Tan CG, Ostrawski S, Bresnitz EA. A preventable outbreak of Pneumococcal pneumonia among unvaccinated nursing home residents in New Jersey during 2001. Infect Control Hosp Epidemiol. 2003;24:848–52.
72. Hoti F, Erasto P, Leino T, Auranen K. Outbreaks of Streptococcus pneumoniae carriage in day care cohorts in Finland - implications for elimination of transmission. BMC Infect Dis. 2009;9:102.
73. Alexander TW, Inglis GD, Yanke LJ, Topp E, Read RR, Reuter T, et al. Farm-to-fork characterization of Escherichia coli associated with feedlot cattle with a known history of antimicrobial use. Int J Food Microbiol. 2010;137:40–8.
74. Li S, Eisenberg JNS, Spicknall IH, Koopman JS. Dynamics and control of infections transmitted from person to person through the environment. Am J Epidemiol. 2009;170:257–65.
75. Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, et al. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog. 2011;7:e1002158.