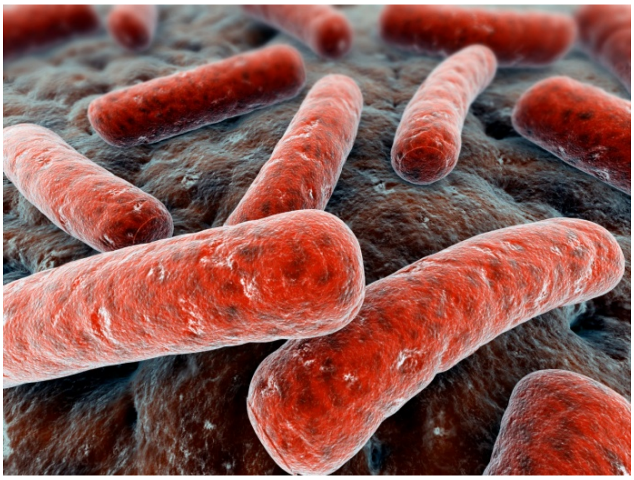
HOUSES OF PARLIAMENT
PARLIAMENTARY OFFICE OF SCIENCE & TECHNOLOGY

POSTNOTE

Number 446 October 2013

# Antibiotic Resistance in the Environment



Disease causing bacteria are becoming increasingly resistant to antibiotic drug treatment. Diseases once controlled by antibiotics are re-emerging as serious risks to human health. This POSTnote outlines the hazards posed by resistant bacteria, the sources of resistance in the environment and measures that may reduce these risks globally.

## Overview

- The presence of resistant bacteria in the environment has been rising because of increased antibiotic use in humans and animals.[25-28]
- Resistant bacteria from human and animal origin enter aquatic and terrestrial environments. Manufactured antibiotics entering the environment create conditions for the proliferation of resistant bacteria.
- Resistant bacteria can be passed between humans, animals and the environment. It is difficult to quantify the risks associated with each of these routes because of the complexity involved.
- The current rise in antibiotic resistant bacteria is a global problem that would require international action to reverse.[25]

## Background

Antibiotics have revolutionised health care. For example, penicillin has saved tens of millions of lives since its discovery.[1] However, the life-saving role of antibiotics is threatened by the emergence of antibiotic-resistant 'super-bugs'.[2-4] The G8 science ministers meeting in 2013 highlighted antibiotic resistance as one of the top threats facing humanity.[5] The difficulty in tackling the global spread of resistance was also highlighted in a World Health Organisation (WHO) report which suggested that no single factor, or isolated intervention, would prove successful in reducing the threat.[6]

### Antibiotic Resistant Bacteria

Antibiotic resistance is a natural phenomenon that has been present in bacteria in the environment for millennia.[7,8] However, the accumulation of manufactured antibiotics in the environment creates the conditions for proliferation of resistant bacteria,[9,10] through the processes explained in Box 1. Resistance is most likely to arise and persist in locations regularly exposed to antibiotics and with poor sanitation.[16-21] The main factors contributing towards resistance vary between countries, for example key problems include self-prescription in India,[22] unregulated pollution in developing countries, such as Cuba,[23] and antibiotic use in animal growth promotion in the USA.[88]

Once resistance is present it can be passed between distantly related species of bacteria and quickly disseminate around the globe.[25] The environmental spread of resistance is primarily governed by two factors:

- the release of substances into the environment that promote resistance
- the release of antibiotic resistant bacteria directly into the environment.[16-21]

**Box 1. How Antibiotic Resistance Develops and Spreads**

Antibiotic exposure promotes resistance by favouring mutations that confer antibiotic resistance in bacteria. These genetic changes can decrease cell wall permeability, preventing antibiotics from entering the bacteria; produce 'efflux-pumps' which actively remove antibiotics from the bacteria; and produce enzymes that destroy antibiotics (see below). Once resistance has arisen the use of antibiotics promotes the proliferation of resistant bacteria. The genes conferring resistance can then be transferred between bacterial species through a process called horizontal-gene transfer.

**Resistant Enzymes**

Of major concern is the emergence of two enzymes called NDM-1 (New Delhi metallo-beta-lactamase-1)[11] and CTX-M beta-lactamase. NDM-1 provides resistance to the antibiotics cephalosporin and carbapenem and CTX-M provides resistance to cephalosporins. The genes responsible for producing these two enzymes have successfully transferred between different bacterial species.[12-14] It has been suggested that if NDM-1 were transferred to a highly contagious bacterium there could be a pandemic against which modern antibiotics would be ineffective.[15]

In many countries antibiotic use in human medicine has been recognised as a major factor contributing towards the rise in resistance (POSTnote 416),[13,15,24] especially in developing nations where drugs regulation is poor and over-the-counter medication is readily available.[6,22] However, scientific evidence suggests that environmental factors are also contributing towards the rise in antibiotic resistance.[16-21,25-27,72] These factors include (1) antibiotics and resistant bacteria accumulating in the waste water treatment process, (2) the release of biocides, antibiotics and resistant bacteria into the environment, (3) the use of antibiotics in agriculture and (4) the direct animal to human transmission of resistant bacteria.

## Environmental Sources of Resistance

There has been an increase in resistance to antibiotics in soil bacteria since antibiotics started to be manufactured in the 1940s.[28] There are two reasons for this:[25-27]

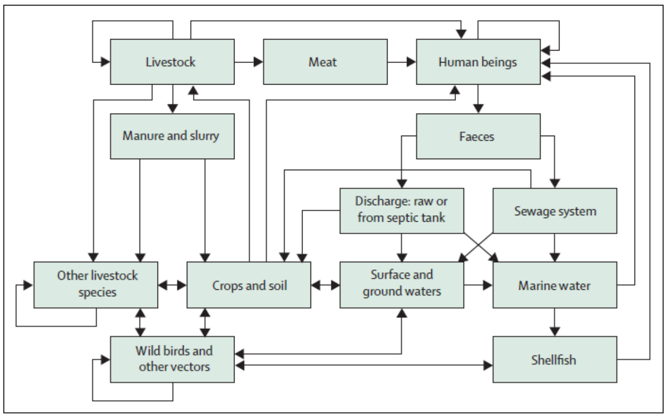
- Resistance is present within bacteria in the environment through exposure to naturally occurring antibiotics. However, the proportion of bacteria with resistance is increased through exposure to manufactured antibiotics.
- Antibiotics and resistant bacteria from human and animal origin directly enter terrestrial and aquatic environments, such as soil, marine areas and surface waters.

Figure 1 provides a simplified summary of the complex pathways involved in the environmental spread of resistance.

### Waste Water

Antibiotics and resistant bacteria from human sources have been detected in all stages of the sewage treatment process, including in treated water released to the environment[29-31] and sludge applied to farmland.[32,33] For example, the fluoroquinolone group of antibiotics, despite degrading in the environment once exposed to sunlight, have been detected on farmland.[21,34-35] Laboratory studies identified human sewage sludge applied as fertiliser as the main source.[35] The highest concentrations of antibiotics and resistant bacteria have been recorded in effluent released from hospitals and drug manufacturing sites in developing countries (Box 2).[36,37]

**Figure 1. Links Between the Sources of Resistance[16]**




**Box 2. Antibiotics in Waste Effluent**

Hospitals and drug manufacturing sites often have the highest concentrations of antibiotics in their effluent, especially in developing countries where the majority of drugs are manufactured. One waste treatment plant in India, receiving effluent from 90 drugs manufacturing companies, was found to release 45 kilograms of the drug ciprofloxacin into the nearby river each day.[36] This equates to 45,000 daily doses. There is also evidence of untreated waste being disposed of directly into water bodies in India. Concentrations of penicillin and other antibiotics similar to those shown to promote resistance have been identified in rivers in China.[37] These sources of antibiotic pollution are of global concern.

*Biocides*

Whereas antibiotics are types of medication designed to kill bacteria, biocides are substances used to control a wide range of micro-organisms, including fungi, viruses and algae, in different environments. They are used as antiseptics, disinfectants and preservatives. They are present in a range of consumer, healthcare, food and industrial products. There is evidence that microbial exposure to biocides can give rise to resistance to antibiotics.[61,62] This has been demonstrated within laboratory settings for a number of biocides, notably triclosan,[61,63] but environmental studies remain scarce. Triclosan is a commonly used biocide present in deodorant, toothpaste and cleaning products.

Biocides can reach much higher concentrations than antibiotics in wastewaters and in some river water. In some waste effluent the concentration is as high as that shown to promote resistance in the laboratory.[61-64] There is also evidence that heavy metals such as calcium and zinc, which are often detected in high concentrations in waste effluent and sludge, may have the ability promote antibiotic resistance in the environment.[65-67]

### Agriculture

Globally around 70% of antibiotic use is in agriculture.[86] Antibiotics are used to treat individual animals, prevent disease (prophylaxis, regulated in the UK) and to promote rapid growth (banned in the EU since 2006). In the UK, the veterinary use of antibiotics is not monitored.[38] However, it is estimated that 30% of antibiotics are used in veterinary medicine,[39] 87% of which is in food-producing animals.[40]

A proportion of antibiotics provided to livestock enters the environment via urine and faeces.[9,10,16,41,42] The quantity of antibiotics excreted and their persistence in the environment is drug dependently. For some types of antibiotic up to 90% of a dose can be excreted in urine and 75% in faeces.[84,85] Field studies on experimental farms have confirmed that crops, such as wheat, lettuce and carrot can take up substantial amounts of antibiotics by the roots.[43,84] However, there is a lack of studies on the fate of antibiotics once in the environment and subsequent hazards posed to humans. There is greater understanding of the link between antibiotic use and resistant bacteria in the environment. Farms in the USA have been shown to have increased numbers of resistant bacteria within their lagoons when antibiotic use is increased.[41] Lagoons are then used for crop irrigation and could provide a direct route for contaminating food with resistant bacteria.[41,42] Assessing the risk from resistant bacteria on UK farms would require relevant studies and monitoring.

*Resistant Bacteria in Livestock*

Resistant bacteria are being transmitted from livestock to humans both directly and via contaminated food.[43-55,84,85] Transmission from humans to animals (from owners to pets) also occurs.[87] A 2008 European Food Safety Authority (EFSA) report stated that:[44]

- the main source of resistant *Salmonella* and *Campylobacter* in humans is food, such as poultry
- animal-derived products are a potential source of MRSA in humans
- cattle is a major source of resistant *E. coli* that may colonize humans via contaminated meat. *E. coli* bacteria in the faeces of cattle in the UK and elsewhere have been shown to contain the CTX-M beta-lactamase enzyme giving rise to resistance to critically important antibiotics[73,74]
- there is potential for food handlers to contaminate food during preparation, as already detected for MRSA and resistant *Shigella*
- avoparcin (an antibiotic of last resort) used in livestock, particularly pigs, was linked to increased resistance to a chemically similar drug, vancomycin, in the life-threatening human pathogen, *Enterococcus faecalis*.[73] Avoparcin was banned for use in farming in the EU in 1997.

There is also evidence of direct animal to human transmission of resistant bacteria. For example, MRSA is usually acquired from human-to-human contact in clinical environments or from visiting countries with a high incidence of MRSA.[45] However, livestock-associated MRSA can be responsible for human cases, as has been shown in Germany.[46-48] There is also evidence of cattle-to-human transmission in Denmark and the UK,[49-52] where cattle farmers are twenty times as likely to carry MRSA as other members of the public.[53] Further evidence of resistant bacteria being passed from animals to humans comes from a study in the USA, where farm workers on intensive farms carried significantly more bacteria with resistance to multiple antibiotics than workers on antibiotic free farms.[54] There is also evidence that resistant bacteria can be inhaled in dust particles released from intensively reared animals.[75] A recent review by the World Economic Forum suggested that resistant bacteria in livestock may potentially cause shortages of food due to untreatable infections in livestock.[55]

### Aquaculture

Aquaculture in the UK has seen significant reductions in antibiotic use. Currently less than 1% of antibiotics are sold for use in aquaculture.[56] However, developing nations continue to use high levels of antibiotics. A review on global antibiotic use in aquaculture identified:[57]

- prophylactic use of medically important antibiotics, such as tetracycline
- that genes for resistance have arisen in aquaculture and been transmitted to animal and human pathogens
- that fish pathogens are one potential route for *E. coli* transmission to humans
- that a strain of resistant *Salmonella* identified in human populations around the world is believed to have originated from aquaculture systems in the Far East.[58]

In the UK, vaccines and legislation have reduced antibiotic use, but a large proportion of consumed fish are imported from non-EU countries. For example, 40% of fish produced in China is bought by EU countries.[16] Unregulated antibiotic use in aquaculture systems in China, and other developing nations, may have global implications for animal and human health.[57]

### Wildlife

Recent scientific publications suggest that wild animals could be involved in the environmental spread of resistant bacteria.[9,10,16] A study conducted in a remote location in Finland found a near absence of resistant bacteria in faeces of wild animals (moose, deer and vole),[60] while a study conducted in the Wirral, UK, found high levels of resistant bacteria in the faeces of forest rodents.[59] This suggests that UK wildlife is being exposed to antibiotics and resistant bacteria of human origin,[16] indicating that wildlife could be vectors in the environmental spread of resistance.[9,10] This is likely to vary between locations.

## Policy in the UK

An overview of UK and international policy is summarised in Box 3. At present, there are no discharge standards for antibiotics and resistant bacteria entering the environment. The Priority Substances listed in Annex X of the Water Framework Directive, which sets safe discharge standards for hazardous substances entering the environment, does not include antibiotics or the majority of biocide compounds (however, see Biocidal Product Regulation EU 528/2012).[78]

The Department of Health (DH) has recently published a five-year cross Government Antimicrobial Resistant Strategy and Action Plan, which covers use of antibiotics in both human and animal medicine.[72] While the report has been welcomed by groups such as the RCVS (Royal College of Veterinary Surgeons), it has been criticised for not addressing the scale of the problem by farming groups such as the Soil Association.[81] There have been a number of

**Box 3. Antibiotic Regulation in Agriculture and Food in the UK**

In the UK, the use of medicines in agriculture and aquaculture is regulated by the Veterinary Medicines Directorate (VMD). Antibiotics should only be administered by registered veterinary surgeons. Once medication has been administered there is a period during which the animal cannot be slaughtered for food or its products enter the food chain. Best practice guidelines are available from the Responsible Use of Medicines in Agriculture Alliance (RUMA). The safety levels of antibiotics and bacteria in food for human consumption are monitored by the Food Standards Agency (FSA).

**International Regulation**

The World Organisation for Animal Health (OIE) is the intergovernmental body for improving animal health worldwide. Among other roles it advises on animal medication policy, antibiotic use and controlling the spread of resistance campaigns by groups such as the Soil Association,[68] Farmers Weekly,[69] Sustain[70] and the Sustainable Food Trust[71] calling for reductions in antibiotic use and an end to antibiotic use in healthy animals. Additionally, the poultry industry has introduced self-imposed restrictions in antibiotic use.

## Alleviating the Risk of Resistance

In the past, resistance to antibiotics was less of a problem because the emergence of resistant bacteria was followed by the development of new antibiotics. However in recent decades fewer new classes of antibiotics have been developed and none are currently in production (Box 4) (POSTnote 311).[77,79]

A number of reports and scientific publications have considered the risk of antimicrobial resistance and provided recommendations on increased monitoring and other courses of action.[3-6,16,72,76,80]

### Antibiotics and Biocides

*Use in Humans*

Campaigns have already reduced the inappropriate use of antibiotics in human medicine. However, further reductions are required and recent reviews have suggested: [3-6,16,72,76,80]

- Antibiotics of last resort should only be used when necessary. This reduces the likelihood of resistance developing and will help reserve critically important drugs. While this is often the case in the UK it is not adhered to in many developing countries.
- Access to and use of surveillance data should be improved. [72]
- There should be increased development of alternative therapies, i.e. bacteriophages (virus that kill bacteria). [72]
- Improving public education on appropriate antibiotic use.
- Use of degradable antibiotics that do not persist in the environment is increased.
- Pharmaceutical products are soured from ethical companies which do not pollute the environment with antibiotics.
- Increased focus on the Biocidal Product Regulation (BPR, Regulation EU 528/2012 effective from Sept 2013) that requires evidence that a biocidal product will not give rise to microbial resistance. [78] The EU will also fund research on the role of biocides on the spread of resistance.[89]

*Use in Agriculture*

Antibiotic use on British farms is already lower than many non-EU countries, including the USA. However, countries such as Denmark and Sweden have substantially lower antibiotic use than in the UK and the French government has introduced plans to reduce antibiotic use over the next five years.[80,82] It has been argued that reductions in antibiotic use could be achieved in agriculture without effecting animal health through: [3-6,16,72,76,80]

- Improved animal husbandry and reduced crowding, which reduce disease outbreaks and thus reliance on antibiotics (see POSTnotes 391 and 404, and DH 5-year strategy).

Box 4. Antibiotic Drug Development

Development of antibiotics has been impeded by a range of factors. These include the low return on investment for development of antibiotics compared to drugs used to treat chronic illnesses and the increased difficulty in discovering new antibiotics once easier ones have been identified. Regulatory burdens have also made the development of new drugs both time consuming and expensive. This is particularly problematic for small pharmaceutical companies with limited funds.

It has been suggested that drug development could be incentivised by increased financial return through extended patents, new regulatory framework based around non-clinical (animal) trials combined with human studies rather and the use of smaller patient numbers. These changes would reduce the time and cost of developing new drugs. Collaborations between academic institutions and pharmaceutical companies could also increase the rate of drug development.

- Improved access to and use of surveillance.[72]
- Improved treatment of animal waste before being applied to farmland to remove antibiotics and bacteria.
- Composting of manures and aeration of slurry greatly reduce bacteria numbers.
- Educating farmers to reduce antibiotic use and provide support to achieve this (available through RUMA).
- Minimising prophylactic use of antibiotics. Prophylactic use is believed to be an ongoing source of resistance by organisations such as the Soil Association.
- Increased research into antibiotic alternatives in food production, such as bacteriophages.
- Reserving medically important antibiotics for human medicine (POSTnote 433). There have been campaigns to reserve fluoroquinolones, which are recognised as vital for human use by the WHO. However, the need for this has been disputed by the Veterinary Medicines Directorate (Box 3).

### Improved Waste Treatment

The waste treatment process has not been designed to prevent antibiotics and resistant genes from being released into the environment. A number of improvements to the waste treatment process have been suggested.[30] These include catalytic oxidation of pharmaceutical compounds. This is a new area of research using iron and hydrogen peroxide to break down antibiotics and compounds such as triclosan.[83] Alternatively, established natural and artificial wetlands have been shown to breakdown high levels of antibiotics and bacteria in their root structures. Over 90% of veterinary drugs can be removed by wetlands.

It has also been suggested that establishment of hospital wastewater treatment plants and additional investment in the infrastructure of treatment plants could reduce antibiotics and resistant bacteria entering the environment. There are currently no limits for the concentration of antibiotics entering the environment; however global safe discharge standards have been proposed.[25]

Endnotes

For references please see:
http://www.parliament.uk/documents/POST/postpn446_Antibiotic-resistance-in-the-environmentreferences.pdf

POST is an office of both Houses of Parliament, charged with providing independent and balanced analysis of policy issues that have a basis in science and technology. POST is grateful to Paul Coleman for researching this briefing, to BBSRC for funding his parliamentary fellowship, and to all contributors and reviewers. For further information on this subject, please contact the co-author, Jonathan Wentworth. 

1 Bud, R, 2007, *Penicillin triumph and tragedy*, Oxford University Press
2 Wise, R *et al*, 1998, *British Medical Journal*, 317: 609-610
3 WHO, *Antimicrobial resistance: a global response*. WHO Bulletin vol.80 No.2
4 Levy, S B, *et al*, 2004, *Nature Medicine*, 10:122-129
5 FCO, 2013, *G8 Science Ministers Statement*
https://www.gov.uk/government/news/g8-science-ministers-statement
6 WHO, 2013, *Antimicrobial resistance*. Fact sheet No 194
http://www.who.int/mediacentre/factsheets/fs194/en/
7 D'Costaf V M, *et al*, 2011, *Nature*. 4: 457-461
8 Bhullar, K, *et al*, 2012, *PLOSone*. 7(4) e34953. doi:10.1371/journal.pone.0034953
9 Gaze, W, *et al*, 2013, *Emerging Infectious Diseases* 19(7), e120871
10 Finley, R L, *et al*, 2013, *Clinical Infectious Diseases* 57(3): 704 – 710
11 Walsh, T R, *et al*, 2011, *Lancet Infectious Diseases* 11: 355-363
12 Potron, A, *et al*, 2011, *Antimicrobial Agents and Chemotherapy* 55: 4896-4899.
13 Canton R, et al, 2009, *Current Opinions in Microbiology* 5: 466 – 475
14 Kumarasamy, K, *et al*, 2010, *The Lancet* 10:597-602
15 Rolan, J M, *et al*, 2010, *Clinical Microbiology and Infection* 16: 1699 - 1701
16 Wellington E M H, *et al*, 2013, *The Lancet* 13: 155-165
17 Rowe-Magnis D A, *et al*, 2002, *International Journal of Medical Microbiology* 292(2): 115 – 125
18 Shakibaie M R, *et al*, 2009, *Journal of Environmental Biology* 30(1): 45 – 49
19 Chee-Sanford, J C, *et al*, 2009, *Journal of Environmental Quality* 38(3) 1086-1088
20 Allen H K, *et al*, 2010, *Nature Reviews Microbiology* 8(4): 251 – 259
21 Knapp, C W, *et al*, 2005, *Environmental Science and Technology*, 39: 9140 – 9146
22 Vikram, P, *et al*, 2005, *JPMG*, 51(1): 9 - 12
23 Graham, D W, *et al*, 2011, *Environmental Science and Technology* 45: 418 – 424
24 Cohen, M K, 1991, *Science* 257: 1050 – 1055
25 Purden, A, *et al*, 2013, *Environmental Health Perspectives* 121(8): 878 – 885
Ulfendahl, M, 2010, *The microbial challenge – An emerging threat to human health, A Joint Programming Initiative on Antimicrobial Resistance*, http://www.era.gv.at/attach/2JPIAntimicrobialResistance2010-05-04.pdf
*EU Joint Programming Initiative on Antimicrobial Resistance*, http://www.jpiamr.eu/
*Action on Antibiotic Resistance* - http://www.reactgroup.org/
*Transatlantic Taskforce on Antimicrobial Resistance (TATFAR)* - http://ecdc.europa.eu/en/activities/diseaseprogrammes/tatfar/pages/index.aspx
26 Kummerer, K, 2004, *Journal of Antimicrobial Chemotherapy* 54: 311 – 320
27 Martinez, J L, 2008, *Science* 321: 365 – 367
28 Knapp, C W, *et al*, 2010, *Environmental Science and Technology* 440: 580 – 587
29 Davidson, P M, *et al*, 2002, *Food Technology* 56: 69 – 78
30 Zhang, T, *et al*, 2001, *Critical Reviews in Environmental Science and Technology* 41: 951 – 998
31 Schluter, A, *et al*, 2007, *FEMS Microbiology Reviews* 31: 449 – 477
32 Gaze, W, *et al*, 2008, *Advances in Applied Microbiology* 63: 249 – 280
33 Henriques, I, *et al*, 2006, *FEMS Microbiology Reviews* 56: 418 – 419
34 Knapp, C W, *et al*, 2008, *Environmental Science and Technology* 42: 5348 – 5353
35 Golet, E M, *et al*, 2003, *Environmental Science and Technology* 37: 3243 – 3249
36 Larsson, J D G, *et al*, 2009, *Regulatory Toxicology and Pharmacology* 53: 161 – 163
37 Li, Y T, *et al*, 2010, *Applied Environmental Microbiology* 76: 3444 – 3451
38http://www.vmd.defra.gov.uk/pdf/Summary_FocusGroups.pdf
39 Pretty, J N, *et al*, 2000, *Agricultural Systems* 65: 113 – 136
40 http://www.vmd.defra.gov.uk/pdf/AMR_overview07.pdf
41 Peak N *et al*, 2007 *Environmental Microbiology* 9: 143 - 151
42 Sarmah A K. *et al*, 2007, *Landbauforschung Völkenrode* 57:25-32
43 Kumar, K. *et al* 2005, *Journal of Environmental Quality* 34: 2082-2085
44 www.efsa.europa.eu/de/efsajournal/doc/765.pdf
45 http://apps.who.int/medicinedocs/documents/s16211e/s16211e.pdf
46 Kock, R, *et al*, *European Journal of Clinical Microbiology and Infectious Disease* 11: 1375 – 1382
47 Kock, R, *et al*, 2010, Eur*opean Surveillance* 15:pii=19688
48 Kock R, *et al*, 2011 *Dtsch Arztebl Int* 108(45): 761–767
49 García-Álvarez, L, *et al*, 2011, *Lancet Infectious Diseases* 11(8): 595 – 603
50 Paterson, G K, *et al*, 2012, *European Surveillance* 17(50): 688 – 695
51 Holems, M A, *et al*, 2011, *Journal of Mammary Gland Biology and Neoplasia* 16(4): 373 – 382
52 Harrison, E M, *et al*, 2013, *EMBO Molecular Medicine* 5(4): 509 – 515
53 Kock, R, *et al*, 2013 *PLoS One* 8:e55040
54 John Hopkins Bloomberg School of Public Health, 2013, *Workers at Industrial Farms Carry Drug-Resistant Bacteria Associated with Livestock*
http://www.jhsph.edu/news/news-releases/2013/heaney-mrsa-farms.html
55 World Economic Forum, 2013. *Global Risks Report* http://www3.weforum.org/docs/WEF_GlobalRisks_Report_2013.pdf
56 VMD, 2010, *Sales of Antimicrobial Products in 2009*
http://www.vmd.defra.gov.uk/pdf/salesanti09.pdf
57 Cabello, F C, 2006. *Environmental Microbiology* 8: 1137-1144
58 Angulo, F J, *et al*, 2005, *Journal of Veterinary Medicine* 51: 374 – 379
59 Osterblad, M, *et al*, 1999. *Nature* 40: 37 – 38.
60 Angulo, F J, *et al*, *Journal of Veterinary Medicine* 51: 374-379.
61 Maillard, J V, 2013, *Microbiological Drug Resistance*, in press
62 Gaze, W H, *et al*, 2011, *ISME* Journal 5 (8) 1253 – 1261
63 Webber, M A, et al, 2008, *Journal of antibiotic chemotherapy* 62: 83 – 91
64 Kümmerer, K, 2009, *Chemosphere* 75(4): 417 – 434
65 Baker-Austin, C, *et al*, 2006. *Trends in Microbiology* 14: 176-82
66 Graham, D W, *et al*, 2011. *Environmental Science and Technology* 45: 418-424.
67 Knapp, C W, *et al*, 2011, PLoS One. 6(11): e27300
68 Soil Association, 2013, *Government must act to Reduce Farm Antibiotic Use*
http://www.soilassociation.org/news/newsstory/articleid/5069/government-must-act-to-reduce-farm-antibiotic-use
69 Farmers Weekly, 2013, *Safe use of Medicines Campaign: Dairy Farm's View*
http://www.fwi.co.uk/articles/05/04/2013/138459/safe-use-of-medicines-campaign-dairy-farmer39s-view.htm
70 Sustain, 2014, *Save our Antibiotics*
http://www.sustainweb.org/foodandfarmingpolicy/save_our_antibiotics/
71 Sustainable Food Trust, 2013, *Antibiotic Resistance*
http://sustainablefoodtrust.org/articles/in-the-news-antibiotics-resistance/
72 DH, 2013, *UK 5 Year Antimicrobial Resistance Strategy* 2013 to 2018
https://www.gov.uk/government/publications/uk-5-year-antimicrobial-resistance-strategy-2013-to-2018
73 Witte, W, 1997, *Dtsch Med Wochenschr* 122: 111
74 Snow, L C, *et al*, 2012, *Preventative Veterinary Medicine* 106(3-4): 225 – 234
75 Harder, B, 2003, *Science News* 164:5
76 Annual Report of the Chief Medical Officer, 2013, *Infections and the Rise of Antimicrobial Resistance*
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/138331/CMO_Annual_Report_Volume_2_2011.pdf
77 Bax, R, 2012, From Coates, *ARM, Antibiotics Resistance, Handbook of Experimental Pharmacology*, 2012, Springer
78 ECHA, 2012, *Biocidal Products Regulation* (BPR, Regulation (EU) 528/2012)
http://echa.europa.eu/regulations/biocidal-products-regulation
79 Piddock, L J C, 2012, *Lancet Infectious Diseases* 12: 249 – 253
80 Wielenga, P, *et al*, 2012, *Combating the risk of antibiotic resistance in animals for the benefit of human health in Denmark*, Report for the International Risk Governance Council, Denark
81 Farming UK, 2013, *Government Strategy 'will not stop excessive use of antibiotics'*
http://www.farminguk.com/news/Government-strategy-will-not-stop-excessive-farm-use-of-antibiotics-_26409.html
82 French Government: EcoAntibi, 2012, *National Action Plan for the Reduction of the Risks of Antibiotic Resistance in Veterinary Medicine*
http://agriculture.gouv.fr/IMG/pdf/130208PlanABR-GB-2012-BD_cle8786a1.pdf
83 Chi, G. T, *et al*, 2013, *International Journal of Chemical Engineering*
http://www.hindawi.com/journals/ijce/2013/760915/
84 Sarmah, A K, *et al*, 2006, *Chemosphere* 65: 725 – 759
85 Halling-Sorenson, B, *et al*, 2005, *Environmental Toxicology and Chemistry* 24: 802 – 810.
86 Mellon, M, *et al*, 2001, *Nucleus* 23: 1 – 3
87 Weese, J, S, *et al*, 2006, *Veterinary Microbiology*: 115(1-3): 148 – 155
88 CDC, 2013, *Antibiotic / Antimicrobial Resistance Threat Report 2013*
http://www.cdc.gov/drugresistance/threat-report-2013/
89 Scientific Committee on Emerging and Newly Identified Health Risks, 2010, Research strategy to address the knowledge gaps on the antimicrobial resistance effects of biocides
http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_028.pdf