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SALMONELLA TYPHIMURIUM DEFINITIVE TYPE (DT) 104

A multi-resistant *Salmonella*

ILSI
International Life Sciences INSTITUTE

REPORT

Prepared under the responsibility of the
ILSI Europe Emerging Pathogen Task Force

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Report on *Salmonella* Typhimurium definitive type (DT) 104: a multi-resistant *Salmonella*

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A multi-resistant Salmonella

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JULY 2000

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EXECUTIVE SUMMARY

- *Salmonella* Typhimurium DT104 is a widespread and internationally important human and animal pathogen.

- The clinical manifestations of gastrointestinal infection may be more severe with DT104 although there is no evidence that it is more invasive than other *Salmonella* spp.

- Isolates of the epidemic strain of DT104 can show resistance to up to nine different antibiotics, although resistance to five is more common. Resistance, with the exception of that to trimethoprim, is chromosomally mediated.

- DT104 may have higher tolerance to heat and acid than many other *Salmonella* but this is not believed to be significant at the practical level.

- DT104 is not a “super bug” and can be destroyed by pasteurisation or proper cooking, for example. Its apparently greater tolerance to heat may reduce margins for error, however.

- In common with other foodborne pathogens, DT104 can mount stress responses to conditions common in food processing. DT104 can also form long, multi-celled filaments at refrigeration temperatures or under conditions of lowered water activity.

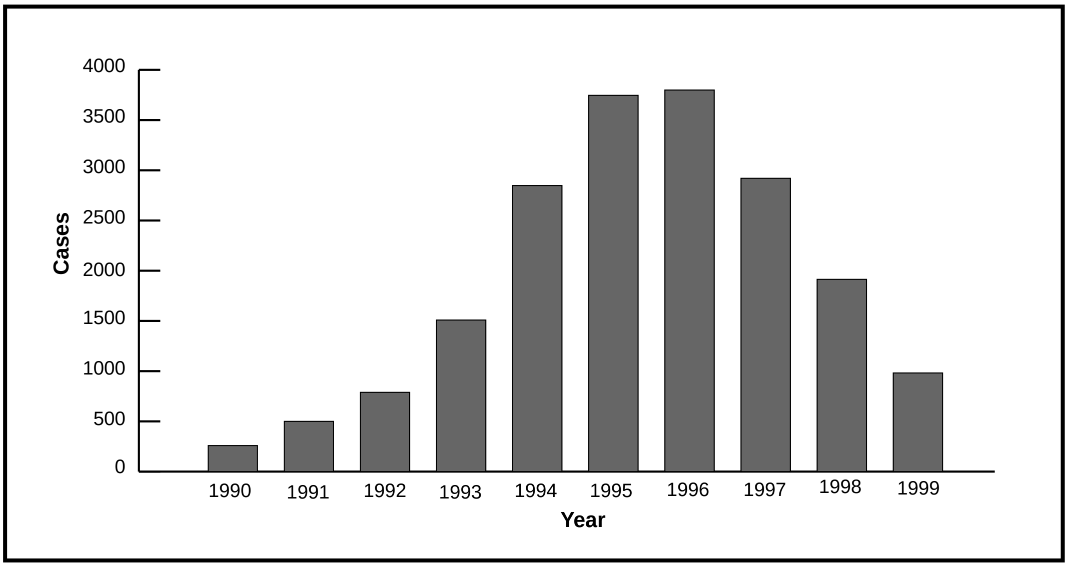
- For the proper control of DT104 and similar pathogens it will be necessary to:

  improve farm hygiene and bio-security; improve animal husbandry and herd and flock health programmes; limit the use of antibiotics in agriculture; enhance surveillance with definitive typing of zoonotic pathogens from animals, foods and humans; and maintain strict hygiene in food processing and commercial and domestic kitchens.

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS OF *S.* TYPHIMURIUM DT104 INFECTION

Salmonella Typhimurium definitive type (DT) 104 is an internationally important human and animal pathogen. It is widespread in Western and Eastern Europe, North America and the Middle East and most isolates of this bacterium have been shown to be resistant to at least five antibiotics. DT104 rose to prominence as a human pathogen in 1990 in Western Europe, although antibiotic-sensitive strains were occasionally isolated from human cases before that time. Data are presented on England and Wales in Figure 1, as these are indicative for much of the rest of Europe. The first reported human isolate of multi-resistant DT104 in the United States (US) was in 1985, though the bacterium did not become widespread in the US until the mid 1990s (Glynn *et al.*, 1998). Recent work has demonstrated that the 1985 isolate is indistinguishable from US DT104 isolates from 1996-98 and the molecular structure of the resistance genes is also identical. The first isolation of multi-resistant DT104 in the UK was in 1984 from seagulls and parrots. The latter were imported from the Far East.

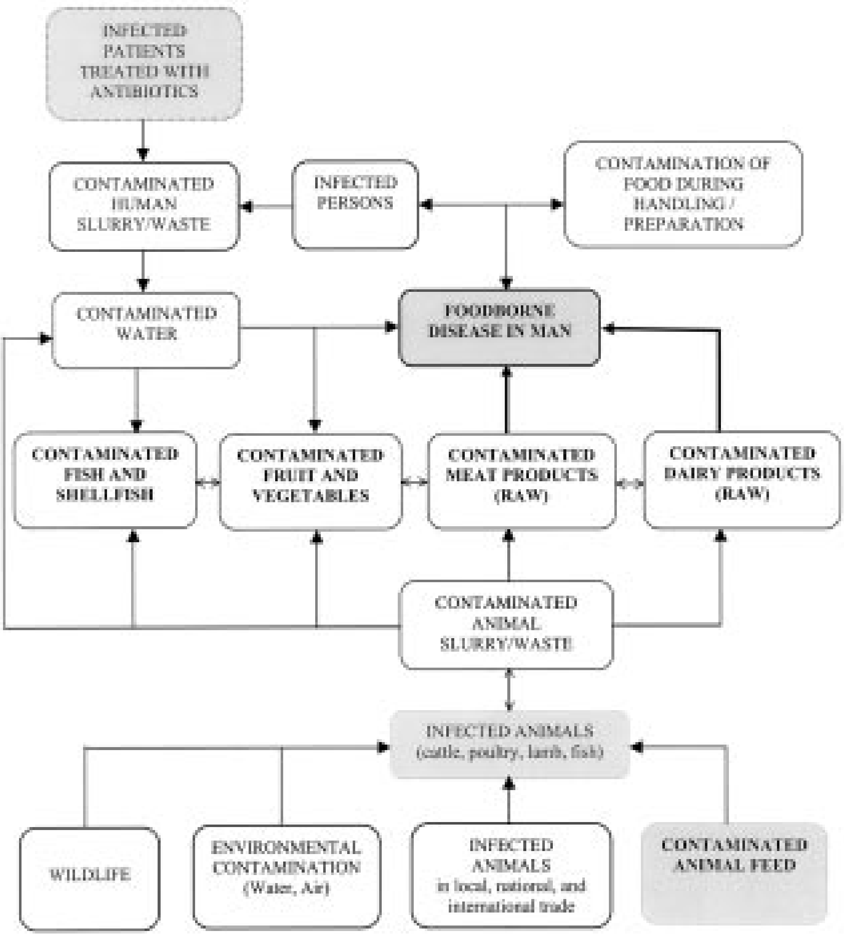
Figure 1: Confirmed human cases of S. Typhimurium DT104 infection in England and Wales, 1990-1999.



DT104 infection in humans is an example of a zoonosis, i.e. an infection acquired from animals. There are many potential routes of transmission and these are illustrated in Figure 2, which outlines the ecology of DT104 in the food chain. The bacterium was originally associated with cattle, where it caused a severe illness, but spread to other food animals, including pigs, where infection may also have severe consequences, and to sheep and poultry. Its association with such a range of animals has resulted in the identification of many vehicles of infection. A case-control study of sporadic cases in England and Wales in the early 1990s revealed that eating sausages, paté and chicken outside the home were associated with illness (Wall *et al.*, 1994). There have also been many outbreaks caused by DT104 reported from Europe, the Middle and Far East, and North America. Vehicles include unpasteurised milk and cheese made from it, undercooked liver, cooked meat and improperly pasteurised milk. Infection can also result from either direct or indirect contact with infected animals (Fone and Barker, 1994).

The case-control study of Wall *et al.* (1994) found that infection with *S.* Typhimurium DT104 was apparently more serious than that caused by other non-typhoid *Salmonella* spp. A greater percentage of those infected were likely to be admitted to hospital (36%) and mortality was higher (3%). The relatively small number of cases in this study may mean that the data were not wholly representative of the pathogenicity of the bacterium and that these results may not be broadly applicable. Reports from recent outbreaks do support the above observations, however. In a pork-associated outbreak in Denmark, 8 of 25 (32%) cases had bloody diarrhoea. In a larger outbreak in the USA, where raw milk cheese was the vehicle of infection, 39 of 54 cases (72%) reported bloody diarrhoea (Villar *et al.*, 1999). Despite these observations on the apparently more serious nature of DT104 infection, it does not seem that the bacterium is any more invasive than other *Salmonella* spp. in human cases. A recent study at the PHLS Laboratory of Enteric Pathogens (LEP) found that the incidence of bacteraemia caused by DT104 was no different from that with *S.* Enteritidis, for example, at around 1.3%. In contrast, the incidence of bacteraemia in people infected with *S.* Dublin is around 40% (H. Smith – personal communication). Irrespective of these findings, DT104 is an important human pathogen that poses particular challenges for the food and agricultural industries and the regulatory authorities.

Figure 2: The ecology of Salmonella Typhimurium DT104 in the food chain.



This figure shows the complexity of *S.* Typhimurium DT104 in the food chain.
Prevention of foodborne salmonellosis by this organism will be achieved by breaking the routes of contamination shown by the arrows. Development of new antibiotic resistant strains will be prevented by restricting the use of antibiotics both prophylactically and therapeutically (shaded boxes).

ANTIBIOTIC RESISTANCE

Patterns in DT104 and other Salmonella spp.

The antibiotic resistance pattern shown by DT104 is dynamic. For example, the first population of isolates from human cases in England and Wales was resistant to five antibiotics:- ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracyclines. These resistances were the result of chromosomal mutations. The next population to appear showed resistance to trimethoprim in addition to the previously mentioned antibiotics and this was plasmid-mediated. In recent years, a clone of DT104 isolates, which reduce susceptibility to fluoroquinolones, has also become important in both animals and man. Some isolates of DT104 from human cases in England and Wales have been found to be resistant to up to nine different antibiotics. These resistance patterns are broadly similar to those of DT104 isolates in the US. The National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria reported that, in 1998, 51% of *S.* Typhimurium isolates were resistant to at least two antibiotics. Fourteen percent of *S.* Typhimurium isolates were also either resistant to or had reduced susceptibility to ciprofloxacin (a fluoroquinolone). Glynn *et al.* (1998) examined multi-antibiotic resistance in *S.* Typhimurium. Their work demonstrated that, in 1979-1980, 1% of isolates of *Salmonella* spp. showed resistance to five or more antibiotics. By 1996, this figure had increased to 34%. They surmised that almost all of these would have been DT104 since a year earlier 91% of resistant isolates were identified as either DT104 or closely related phage types included in the DT104 complex (Glynn *et al.*, 1998). The current pandemic of DT104 infection is reminiscent of an earlier outbreak, caused by *S.* Typhimurium DT204C, which caused severe infection in young bovines and was also an important human pathogen. Isolates of this *Salmonella* spp. were also resistant to a wide range of antimicrobial agents (Wray *et al.*, 1986).

A few other *Salmonella* serovars show resistance to particular types of antibiotics. For example, 40% of clinical isolates of *S.* Hadar from human cases in England and Wales in 1994 showed reduced susceptibility to ciprofloxacin. By 1998 this percentage had increased to 68% (H. Smith – personal communication). The NARMS data from the US also demonstrates that resistance to single antibiotics is also on the increase in other *Salmonella* serovars. Thus c. 34% of *S.* Enteritidis isolates showed resistance or reduced susceptibility to fluoroquinolone antibiotics. This is probably a consequence of the use of these antibiotics in poultry production.

Multi-antibiotic resistance is, however, rare in *Salmonella* spp. in general, particularly in serovars other than *S.* Typhimurium. In a study of almost 40,000 isolates from both medical and non-medical sources in Scotland, only 0.002% showed multiple resistance (Rankin, 1998). The author concluded that the epidemic spread of *S.* Typhimurium DT104 must be recognised as the clonal spread of a single organism in a manner similar to that of *S.* Enteritidis phage type (PT) 4. This view is supported by work in France, which found that a single clone with a chromosome-borne Class 1 integron was widely and equally distributed among human and animal DT104 isolates. The French clone was genotypically indistinguishable from a multi-resistant DT104 clone found in the UK (Casin *et al.*, 1999).

There is not yet a full understanding of the reason behind why certain *Salmonella* serovars such as *S.* Typhimurium and *S.* Hadar show a much higher prevalence of antibiotic resistance than others. Ridley and Threlfall (1998) demonstrated that resistance genes in DT104 are in integrons. There is the possibility that the lack of restriction modification systems in *S.* Typhimurium may make it more receptive to the receipt of incoming DNA (Threlfall – personal communication). This view is supported by recent work (Briggs and Fratamico, 1999) which demonstrated that the resistance genes of DT104, both integron- and transposon-mediated, are encoded by a discrete segment of DNA which appeared to be most closely related to plasmids of *Pasteurella* Piscicida and *Vibrio* Anguillarum, two fish pathogens. This has led to the view that the DT104 resistance (R)-type may have evolved in aquaculture, perhaps in Asia (F. Angulo – unpublished).

In bacteria other than Salmonella spp.

It should be borne in mind that many bacteria other than *Salmonella* spp. are capable of becoming resistant to antibiotics. A range of bacteria started showing resistance to penicillin within 12 months of the start of its use in hospitals. Thus, within this short time, isolates of *Staphylococcus* Aureus from patients in a London hospital showed a 3000-fold increase in penicillin resistance (Anon., 1999c). In the 1940s, 95% of isolates of *S.* Aureus were penicillin-sensitive; now 95% are resistant (Anon., 1999c). Over the last 10-15 years there has also been a marked and widespread increase in the number of *S.* Aureus strains resistant to methicillin (MRSA). Vancomycin-resistant enterococci (VRE) have also become important nosocomial pathogens. Both MRSA and VRE may have appeared as a consequence of antibiotic use in hospitals, but vancomycin resistance in enterococci could also be the result of the usage of related antibiotics in agriculture (Anon., 1999a).

DOES *S.* TYPHIMURIUM DT104 SURVIVE FOOD PROCESSING TREATMENTS?

There is understandable concern that the resistance of *S.* Typhimurium DT104 to such a range of antimicrobial agents is accompanied by increased tolerance or resistance to other compounds and to treatments and processes common in food production. Thus, is DT104 a "super bug"? Work is in progress to address this issue in both Europe and North America. Data produced so far indicate that DT104 may have higher heat and acid tolerance than many other food-associated *Salmonella* spp. Provided that foods are properly cooked, or processed and protected from re-contamination post-processing, there is no evidence to suggest that DT104 poses a danger over that of other *Salmonella* spp. It does share, with other *Salmonella* spp. such as *S.* Enteritidis PT4, however, an ability to respond to a range of conditions common in food production and this is considered below.

THE ADAPTABILITY OF DT104 AND OTHER *SALMONELLA* SPP.

The ability of bacteria to respond to changes in their immediate environment is increasingly recognised as an important challenge to the international food industry. Thus in the last ten years we have gained a greater understanding of how bacterial responses to food-related environments enable them to better survive procedures designed to render food safe. For example, exposure of *S.* Enteritidis to egg albumen, which is alkaline, induces the synthesis of heat shock proteins, which help to protect the bacterium from subsequent heat treatment. Similar responses are seen if *Salmonella* are cultured in mildly acidic environments like mayonnaise (Foster and Hall, 1990). Such behaviours are termed "stress responses" and are discussed below. *Salmonella* spp. will respond to changes in either the temperature or pH value of its environment. It is important that this is taken into account when studies on food processing are to be made. In all such work, pre-challenge culture methods, which most closely mimic the conditions likely to be experienced by the bacteria before processing, should be used.

The above responses are largely under the control of the *rpoS* gene in encoded σ^S sub-unit of RNA polymerase which is a major regulator of bacterial stress responses. Cells unable to express this protein show increased sensitivity to starvation, high osmolarity, heat, acid, etc. The changes in tolerance induced by RpoS can be transient and cells will return to "normal" behaviours once the stimulus has been removed.

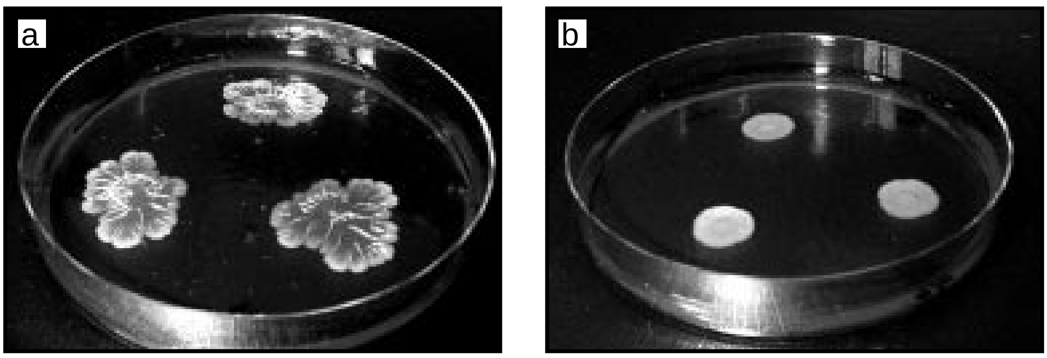
GENETIC VARIATION IN *SALMONELLA* SPP.

In addition to being able to mount stress responses, some *Salmonella* strains are capable of more frequent mutations which may bring about permanent changes in tolerance, resistance and / or virulence (LeClerc *et al.*, 1996). This may be manifested as antibiotic resistance, as in DT104, although this could also be due to the receipt or exchange of genetic material. It does mean that such *Salmonella* populations are in a state of frequent change. These population dynamics can present problems to scientists wishing to study these bacteria and, more importantly, to the food industry. Mutations in the stress-response gene *rpoS* are common in *S.* Typhimurium, as they are in *S.* Enteritidis. The PHLS Food Microbiology Research Unit (FMRU) has recently conducted a study of the tolerance, survival and stress responses of DT104. This work involved an analysis of over 50 isolates of DT104 from human cases, infected animals, foods and the environment. The study revealed that DT104 is very similar to *S.* Enteritidis PT4 in that isolates comprise essentially two populations. The majority has normal RpoS expression and must be regarded as being "wild type", whereas a minority population has mutations resulting in impaired RpoS expression.

SURVIVAL OF DT104 IN FOOD-RELATED ENVIRONMENTS

A comparative study of wild type and *rpoS* mutants of DT104 reveals profound differences in behaviour. Wild type isolates show marked increases in heat and acid tolerance on culture to stationary phase, are capable of extreme persistence on surfaces or in aerosols (see later), and are virulent in animals infected orally. In addition, they can be characterised by a "convoluted" colony morphology on agar plates incubated below 30°C where colonies can resemble those of *Bacillus* spp. (Jameson, 1966). The *rpoS* mutants remain essentially heat- and acid-sensitive, even in stationary phase, survive poorly, are avirulent in laboratory animals infected orally and have a "smooth" colony morphology. These behaviours are not peculiar to DT104 but can be found in other *Salmonella* spp. and in isolates of *Escherichia* Coli O157:H7 where *rpoS* mutations are also common (see "convoluted" and "smooth" morphologies, as in Figure 3a and Figure 3b respectively). It is important that when studies on tolerance and the efficacy of food processing treatments are conducted, representative isolates of the population under study are used. The use of *rpoS* mutants, for example, could lead to the conclusion that treatments are safe because of the inherent sensitivity of the chosen bacterium. A simple check on colony morphology should enable a wild type isolate to be chosen.

Figure 3: (a) Colonies of S. Enteritidis PT4 strain E, which has normal RpoS expression, on Brilliant Green agar (BGA) after 12 days at 25°C. (b) Colonies of S. Enteritidis PT4 strain I, which has impaired RpoS expression, on BGA. Incubation conditions were as in (a).*



** RpoS gene is largely involved in the regulation of bacterial stress responses through encoding of sub-units of RNA polymerase. Cells unable to express this protein show increased sensitivity to starvation, high osmolarity, heat, acid etc... The changes in tolerance induced by RpoS can be transient and cells will return to "normal" behaviours once the stimulus has been removed.*

It is of interest to speculate as to why *rpoS* mutants are so common in both foods and the environment when laboratory studies on tolerance and virulence have shown these bacteria to be at such a disadvantage. There is the possibility that the ability of the bacteria to attach to food matrices (Humphrey *et al.*, 1997) and the inherently protective nature of food and agricultural materials permit the prolonged survival of even those strains of DT104 with markedly lowered tolerance.

From the work undertaken so far on DT104 and the other foodborne pathogens it is possible to conclude that DT104 does not possess a tolerance which compromises proper food production or cooking. What can be concluded is that the acquisition of resistance to a range of antimicrobial agents does not appear to have compromised the physiology of DT104. These bacteria may not pose an exceptional threat to the international food industry. They do, however, slightly lessen the margins of error in food processing and/or cooking. This view is supported by the frequent implication of foods such as cooked chicken, paté and sausages in cases of infection and the fact that only a small degree of under-pasteurisation of milk has led to widespread outbreaks.

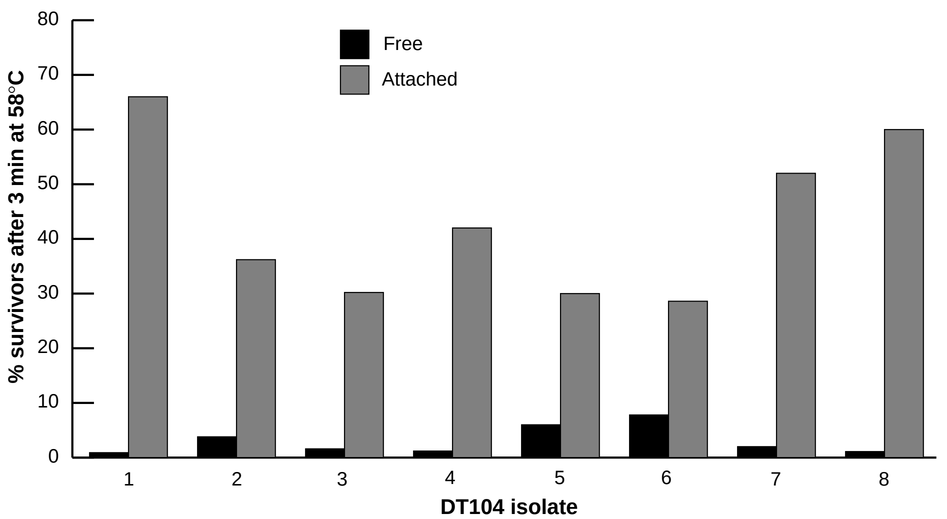
SPREAD AND PERSISTENCE IN THE FARM, THE FOOD FACTORY, AND THE DOMESTIC KITCHEN

Isolates of DT104 are capable of surviving for long periods on kitchen and factory surfaces as they are in the natural environment. In protective materials such as high fat foods, like egg or chocolate, or where blood is present, death rates can be measured in months and small sub-populations may be able to survive for years. DT104 wild type isolates also appear to have the ability, like PT4, to survive in aerosols. This means that they are able to spread easily from one area to another whether on the farm, in the factory or in domestic kitchens. The public health implications of these attributes have yet to be fully explored but are worthy of further investigation. A recent study compared the oral and aerosol routes of infection with DT104 in laying hens. Groups of 15 commercial laying hens were infected with either c. 107 cells of a DT104 isolate by the oral route or with c. 200 cells in an aerosol. With the former group, approximately 1% of intact eggs had *Salmonella*-positive contents. Breast muscle tissues examined 14 days post-infection were sterile. In contrast, infection by the aerosol route resulted in 14% of the contents of eggs being contaminated with *S.* Typhimurium. In addition, 27% of deep muscle tissue samples were *Salmonella*-positive two weeks after infection (Williams *et al.*, 1998; Leach *et al.*, 1999). The reasons for the marked differences between the routes of infection or what governs the ability of DT104 to survive so well in aerosols are not known but are being investigated.

ATTACHMENT TO FOOD MATERIALS

Comminuted meat products, like British fresh sausages, are important vehicles of infection with DT104. If such products are cooked from frozen it is possible to isolate cells of DT104 previously inoculated into the sausage at low levels, even when the sausage appears cooked and manufacturers' instructions have been followed. There is a possibility that this is associated with the ability of DT104 to attach to muscle tissues (Humphrey *et al.*, 1997). In a recent study in the UK, cells of DT104 in stationary phase were mixed with pieces of freshly ground pork. Attachment to these tissues was rapid and resulted in a marked increase in heat tolerance compared to unattached cells (Figure 4). Such attachment is a common feature of Gram-negative bacteria and is more likely to happen immediately after mincing than when tissues have been stored. This behaviour has important implications for the food industry. If pre-formed products are inoculated when cooking/processing is assessed, survival of *Salmonella* spp. may be underestimated because attachment may not be as good under these conditions as during manufacture where muscle tissues are exposed for the first time.

Figure 4: Effects of attachment to pork muscle tissues on the heat resistance of S. Typhimurium DT104.

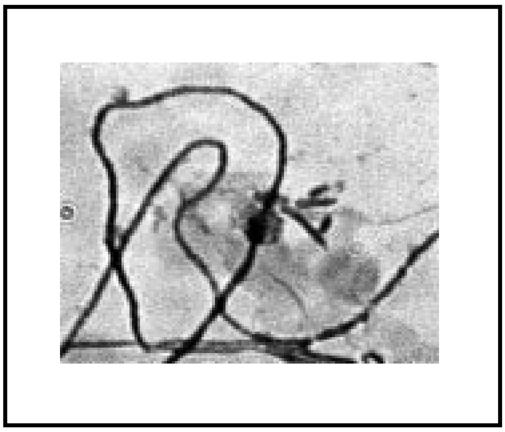


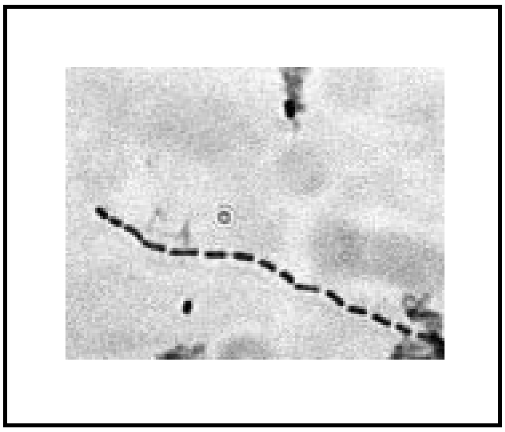
The temperature of 58°C was chosen as this allowed an easier distinction between free and attached cells.

FILAMENTATION OF *S.* TYPHIMURIUM DT104 UNDER CONDITIONS WHICH DO NOT PERMIT "GROWTH"

Storage at low temperature and reductions in the water content of foods are important in the maintenance of food safety and quality. *Salmonella* spp. have been reported to be able to grow at temperatures as low as 7°C, although at this temperature growth rates would be very slow. Until recently, it was generally believed that growth does not occur below this temperature. "Growth" in microbiological terms is usually defined as an increase in cell number and is frequently assessed by using plate counts. It may be necessary for this rather rigid definition to be re-assessed. Recent work has shown that strains of PT4 and DT104, in a manner similar to that of *Escherichia* Coli, can show marked increases in biomass under conditions thought not to permit "growth". Under these conditions, however, cells are unable to divide. This leads to filament formation. For example, when strains of either PT4 or DT104 are held in foods, such as milk or chicken, or laboratory media at 4°C, those with normal RpoS expression form filaments which can exceed 200 µm in length (Phillips *et al.*, 1998). Strains with impaired RpoS expression remain largely unchanged at 4°C. Similar behaviours are seen when PT4 or DT104 cells are cultured in conditions of lowered water activity, such as broth containing 8% NaCl (Figure 5). Interestingly, under these conditions both wild type and *rpoS* mutants can form filaments.

Figure 5: Behaviour of DT104 at low a_w (=available water). Strain held at 21°C in 8% NaCl (a_w 0.95) in nutrient broth for 6 days (left) with the a_w then being raised to 0.99 (right).

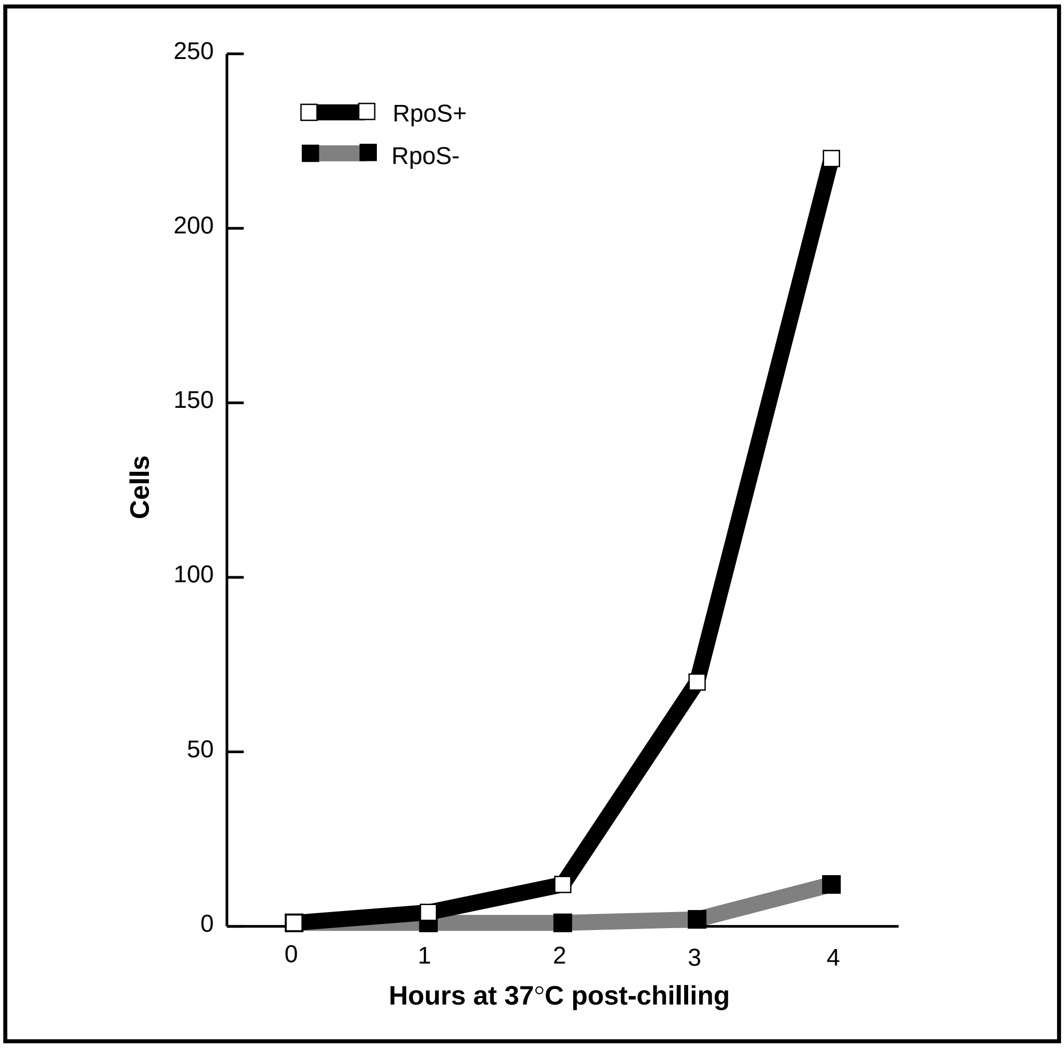




It appears that reductions in either temperature or the levels of available water inhibit septation, probably at an early stage. Filaments contain many copies of chromosome DNA. Studies with chilled cells demonstrated that when temperatures of cultures were raised from 4°C to 37°C the strains which express RpoS, and which form filaments, rapidly complete septation. This will lead to a marked and rapid increase in the *Salmonella* population compared to *rpoS* mutants, which do not filament (Figure 6).

The public health implications of these behaviours have yet to be fully elucidated, but it should be borne in mind that chilled foods are important vehicles in outbreaks of salmonellosis, and also dry snack foods with a lower water activity, such as potato crisps, have also been vehicles of infection. Studies of the sort outlined above broaden our understanding of the many abilities of *Salmonella* spp. such as DT104 to respond to food-related environments.

Figure 6: Increase in cell numbers from a single PT4 cell at 37°C following 14 days at 4°C.



CONTAMINATION OF FOODSTUFFS

Salmonella Typhimurium DT104 continues to be common in food animals and as a consequence of this it is prevalent in the natural environment. In addition to the vehicles of infection detailed above, it has been isolated from a wide range of foodstuffs including raw meat and poultry, milk, raw cereals and vegetables and bi-valve shellfish. It is believed that cereals and vegetables become contaminated as a consequence of fields being fertilised with manure from infected animals, and shellfish become contaminated because of pollution of estuaries. The survival capabilities described earlier in this article are likely to mean that DT104 isolates will be able to persist in soil, water and animal faeces for long periods.

ANTIBIOTIC-RESISTANT BACTERIA ARE NOT A NEW PROBLEM

Antibiotic usage in humans and animals inevitably leads to the appearance of resistant strains of not only the target bacteria but also others occupying the same ecological niche. This latter point may be of particular importance as genetic material can be transferred between different genera of bacteria. Studies in the Netherlands over 20 years ago also demonstrated that bacteria present on chicken carcasses could become transient members of the gut flora of people who handled the raw chickens. Problems of antibiotic-resistant bacteria are not new, although the relatively short half-life of scientific information can create this impression. The World Health Organization (WHO) held a meeting in 1963 to discuss "the public health aspects of the use of antibiotics in food and foodstuffs". At this meeting the conclusion was reached that, whilst it was desirable that antibiotics other than those of medical value should be used for growth promotion, the committee accepted that it was not possible to achieve that at the time and concluded: "Provided that the use of antibiotics is intelligently exploited, the advantages to the consumer are great and the hazards few or non-existent". This view was largely overtaken by events, and the rapid rise in resistance in bacterial pathogens to antibiotics such as penicillin and tetracycline led to a change in view. In the UK in 1969 the "Swann Report" recommended that the use of certain antibiotics as growth promoters in animal production should be discontinued and that there should be antimicrobials dedicated to human medicine only. The appearance of enteropathogens, such as *S.* Typhimurium DT104 at present and DT204C in the past, shows that much remains to be achieved.

Food manufacturers receiving raw ingredients must expect them, on occasions, to be contaminated with DT104. What preventative measures are appropriate to deal with this important human pathogen?

WHAT CAN BE DONE TO PREVENT ANTIBIOTIC RESISTANCE SPREADING?

It must be expected that resistance to antimicrobial agents in bacteria is a natural consequence of their use. In human medicine, treatment is focused at the individual patient level. In animal medicine this may also be the case, particularly with large animals. In the last 2-3 decades there has been a marked intensification of animal production, particularly with pigs and poultry. In this situation it is normal practice to treat all animals in the herd/flock, even if only a proportion are infected. In these cases, antibiotics are administered in either water or feed. Certain classes of antibiotics may still also be used as growth promoters being incorporated into feed and fed continuously during periods of the production cycle. In public health terms, the mass treatment of animals is undesirable as it will be difficult to control the levels of antibiotics received by individual animals and this may lead to consumption of less than optimum dosages. In addition, the high stocking densities will also facilitate the spread of antibiotic-resistant strains as it does that of pathogenic bacteria. The continued cost pressures faced by agriculture mean that it is unlikely that production systems will become less intensive. It is important, therefore, to identify ways in which animals can be reared in high numbers without the excessive use of antimicrobial agents.

Problems will be exacerbated when the antimicrobials are in widespread use, such as the tetracyclines and latterly the fluoroquinolones. The central tenet to this discussion document is that the use of antibiotics for either prophylaxis or for growth promotion in food animals will generate resistant bacteria more rapidly and to a greater degree than when usage is targeted at the individual patient or animal. This problem is not confined to certain *Salmonella* spp. MRSA and VRE were mentioned earlier. Surveys in Europe have shown that VRE was isolated from 79% of poultry carcasses and 59% of live chickens respectively (quoted in Salyers, 1999). In many countries marked increases in resistance to fluoroquinolones in *Campylobacter* spp. have also been reported (Anon., 1999a). This is thought to be due to the widespread use of the antibiotics in poultry production.

There is increasing international concern over these issues and expert groups have been convened in a number of countries or trade organisations to address this problem. In the UK, for example, the House of Lords Science and Technology Select Committee recently produced a document entitled "Resistance to Antibiotics and Other Antimicrobial Agents". Amongst the many statements made in the report, the one which said: "This enquiry has been an alarming experience, which leaves us convinced that resistance to antibiotics and other anti-infective agents constitutes a major threat to public health and ought to be recognised as such more widely than it is at present", encapsulated the committee's concerns. Another UK report, this time from the Advisory Committee on the Microbiological Safety of Food (ACMSF) and entitled "Microbial Antibiotic Resistance in Relation to Food Safety" has also just been published. In May 1999, the European Commission Scientific Steering Committee gave an "Opinion on Antimicrobial Resistance" (Anon., 1999a). Although these various expert working groups had different memberships and were presented with different bodies of evidence, they came up with essentially the same conclusions, which can be best illustrated by reference to the European Commission Scientific Steering Committee. The Committee made a number of recommendations. These were:

a. The use of antimicrobials should be prudent.
b. There should be strategies for the prevention of infection and the containment of resistant organisms.
c. There should be new modalities of prevention and treatment of infection.
d. The effects of interventions, with antibiotics, or otherwise, should be monitored.

The prudent use of antimicrobial agents

The principal thrust of the above opinion is that antimicrobials should be used prudently. It is difficult to obtain an accurate figure for antibiotic usage. It is estimated that, in the USA, 2000-2500 tons are used annually, half of which are in animal feed. In Denmark in 1998, 57 tons were used for animal treatment, 49 tons as growth promoters. Clearly, farmers and veterinary surgeons must retain the right to treat sick animals. There is also a powerful economic argument for growth promotion. This can be outweighed by public health concerns and consequently, from 1 July 1999, the use of zinc bacitracin, spiramycin, virginiamycin and tylosin phosphate was banned as growth promoters in the European Union. Avoparcin was banned earlier. The logical conclusion to these approaches is that there be a separation of the antibiotics used in animals, in any form, from those used in humans.

The UK Departments of Health recently issued a document entitled "The Path of Least Resistance" which contained advice for General Practitioners on four things that they could do to reduce the generation of antibiotic resistant pathogens (Anon., 1999b). These were:

- No prescribing of antibiotics for simple coughs and colds.
- No prescribing of antibiotics for viral sore throats.
- Limit prescribing for uncomplicated cystitis to three days in women who are otherwise fit.
- Limit prescribing of antibiotics over the telephone to exceptional cases.

Strategies for the prevention of infection and the containment of resistant organisms

The spread of pathogenic bacteria, including those that are multi-resistant, in hospitals and institutions could be minimised by proper attention to internationally-agreed infection control strategies. Education of farmers, veterinarians and pet owners on disease prevention would also clearly have an effect on the transmission of zoonotic pathogens such as DT104. There is much attention focused on the consumer as a key player in the transmission of foodborne disease. There is no doubt that education measures to improve home hygiene would be beneficial. The problems in controlling the spread of *Salmonella* spp. from contaminated eggs or chicken meat, for example, mean that consumers will always experience difficulties and control is perhaps best achieved on the farm or in the food factory.

New modalities of prevention and treatment of infection in farm animals

There is understandable concern that restricting antimicrobial usage in agriculture will have welfare implications. Recent experiences in Sweden might provide a degree of comfort. In 1986 the Swedish Parliament imposed a ban on all antibacterial growth promoters. This eventually led to a 50% reduction in antibiotic usage in animal production. In the first year after the ban, piglet mortality increased by 1.6% and the incidence of diarrhoea quadrupled. Changes in management practices, however, have brought about a reduction in infection so that the figures are closer to those before the ban (Spring, 1999). Such approaches, which include improvements in farm hygiene, will also lead to a reduction in antibiotic resistance. In Sweden the incidence of streptomycin resistance in *S.* Typhimurium strains isolated from pigs has declined from 78% in 1986 to 17% in 1992-94. Similarly, resistance to tetracycline dropped from 14 to 0% (Anon., 1999c).

Disease protection can also possibly be achieved, in the absence of prophylactic antibiotics, by adopting novel feeding systems. Research from Denmark on pig production suggests that the use of fermented liquid feed, which will have a low pH and improved digestibility, brings about dramatic reductions in levels of potentially pathogenic bacteria. There must be continued recognition that animal and human health can never be entirely separated.

Monitoring of interventions with antibiotics

The international pandemics of human infection caused by DT104, VRE and MRSA have helped to identify the marked differences in surveillance activity between countries. There is a need for a standard approach and for information to be properly shared.

The need for improved, co-ordinated surveillance has been recognised and programmes have begun in Western Europe and North America. In Europe, for example, data on gastrointestinal infections is shared by surveillance organisations using a system termed “Enteronet”.

With respect to DT104, in particular, it is important that isolates of *Salmonella* spp. from human cases are phenotyped (sero- and phage-typed and have standard antibiograms), and that this information be recorded. In addition, standardised molecular typing will contribute to tracing isolates from humans back through the food chain to the animal reservoir. In this way trends in infection can be more readily identified, and this may make it possible to invoke preventative strategies at a comparatively early stage. In addition to targeting zoonotic and other pathogens, the monitoring of indicator food contaminants – such as the enterococci – for changes in resistance patterns has already given a useful indication of possible changes in antibiotic usage and resistance profiles.

SHORT- AND LONG-TERM RESEARCH AND SURVEILLANCE GOALS

There is a need for increased research on:

- Mechanisms of antibiotic resistance and why some bacterial strains show increased resistance.
- Methods of animal production which help to minimise antibiotic usage.
- Persistence of antibiotic-resistant bacteria in food production systems and the environment in the absence of selective pressures.

Salmonella Typhimurium DT104 and other antibiotic-resistant human pathogens present a clear danger to human health above and beyond that of causing infection. A chronology of food scares has damaged consumer confidence in the food industry and the regulatory agencies. Widespread fears, that infections may become "untreatable", will fuel consumer concerns even further. The speed of development of new antimicrobials, particularly those with a broad spectrum, is much slower than the acquisition of resistance. This report has discussed certain possible actions that could serve to lessen the entry of antibiotic-resistant bacteria into the food chain. In the short term, these include improved surveillance, collaboration and reporting; reductions in the use of prophylactic and growth-promoting antibiotics in animal production; research into antibiotic resistance mechanisms and the persistence of resistant bacteria. In the longer term, it is necessary to investigate whether changes in animal production systems such as a reduction in stocking densities and improvements in hygiene can obviate the need for antimicrobials.

The increasingly global nature of food production means that it has never been more vital that information is shared between countries. Geography alone is not sufficient to protect consumers in one country from animal health / production issues in another. There is general agreement that foodborne disease is best controlled by a "farm-to-fork" approach. Everyone has a shared responsibility to protect themselves and their customers from illness. With pathogens such as DT104, risk reduction is most effectively exercised on either the farm or in the food factory and the residual risk in raw products communicated to food processors, caterers, retailers and the general public. Governments have an important role also in this process. Surveillance systems must be comprehensive and open. Frameworks should be created in which information on trends in human and animal disease is shared. Government departments must also provide sound and sensible advice to both consumers and food producers on food safety issues.

There is also a need for all those involved in food production, from government to farmers, veterinarians and food producers, to continue to remember that it is impossible to completely separate agriculture from public health.

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APPENDIX

FACT SHEET *SALMONELLA* TYPHIMURIUM DT104

Summary

The bacterium *Salmonella* Typhimurium DT104 is attracting attention because it is causing increasing numbers of cases of salmonellosis in man and food animals. It now is spreading through raw meat, poultry and vegetables. Some isolates have become resistant to as many as nine different antibiotics. The resistance to antibiotics has probably emerged as a result of the widespread use of antibiotics on farms. The long-term objective must be to eradicate *S.* Typhimurium DT104 from the food chain. This will require concerted efforts by governments and farming communities throughout the world.

The organism and its resistance to antibiotics

Salmonella Typhimurium Definitive Type (DT) 104 is resistant to a wide range of commonly used antibiotics. The epidemic clone is now resistant to as many as nine antibiotics, including ampicillin, chloramphenicol, streptomycin, the sulphonamides and the tetracyclines. Some strains are now appearing which are also resistant to trimethoprim and to quinolone antibiotics such as ciprofloxacin. The disturbing situation here is that the quinolones are a group of antibiotics that are sometimes used in man for the treatment of salmonellosis, including enteric fever. Quinolones are also used for the treatment of salmonellosis in cattle, pigs and poultry. The emergence of all these organisms is judged to have arisen through the misuse of antibiotics in veterinary medicine, both prophylactically and therapeutically. The genes that code drug resistance are carried on the chromosomes of the DT104 so that once a bacterium has acquired resistance it may be very difficult to lose it in the future.

Spread of the pathogen in the food chain

Salmonella Typhimurium DT104 was first associated with cattle but it has spread to a range of food animals, especially pigs, sheep, chickens and turkeys. The organism seems to be invasive in cattle and pigs, where it causes serious losses of animals. As with many other *Salmonella* spp., some infected animals show clear clinical symptoms, but some do not. *Salmonella* Typhimurium DT104 can be spread from farm to farm by raw water, feed and effluent systems. The purchase of infected animals is also important. Once a farm becomes contaminated, it is difficult to eradicate DT104 because it survives well in wet or dry environments. The organism has now been isolated and identified in Austria, Canada, Denmark, France, Ireland, Germany, Italy, The Netherlands, Sweden, the Middle East, the UK and in 50 states in the USA.

Food materials likely to be contaminated

The raw food materials likely to be contaminated are: raw beef, veal, pork, lamb, goat, chickens and turkey; raw eggs; raw milk; raw vegetables and cereals; raw shellfish.

Severity and rising incidence of the disease in man

Although human intestinal illnesses are not normally treated with antibiotics, when treatment is needed, the illness caused by *S.* Typhimurium DT104 is more difficult to treat. The mortality rate is 3%, which is higher than the 0.1% for other *Salmonella* spp. The incidence is rising throughout the world.

Survival and growth of the organism in food

Apart from its multiple resistance to antibiotics, *S.* Typhimurium DT104 shows similar characteristics to other *Salmonella* spp. Thus the pasteurisation processes, which are recommended to destroy “ordinary” *Salmonella* spp., will also destroy *S.* Typhimurium DT104. There is evidence that the organism becomes more heat-resistant when it is attached to muscle tissues.

Control in the food chain

The worst scenario is that the organism will become widespread throughout the food chain with a huge increase in human salmonellosis – as occurred in the early 1990s when *S.* Enteritidis became endemic in poultry flocks. Such a situation will be more difficult to control because, whereas *S.* Enteritidis was confined to poultry, *S.* Typhimurium DT104 occurs more widely. Control in the food chain will require a co-ordinated effort by governments and the farming industry throughout the world. Focused action is being taken in Scandinavia and is planned for the European Union.

Control in the food manufacturing and catering industries

The current advice is that the methods to control common *Salmonella* spp. will also control *S.* Typhimurium DT104. In particular, food manufacturers must operate effective Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Point (HACCP) programmes in order to control growth of the pathogen and prevent build-up within the factory. They should also give safe cooking and handling instructions on packs of raw foods. Catering services must ensure safe cooking and good hygienic practices at all times.

Laboratory isolation

The methods to isolate *Salmonella* spp. from foods will also isolate *S.* Typhimurium DT104 if it is present. However, the food industry will not be able to phage-type the isolates nor carry out antibiotic resistance profiles. If needed, these would have to be done by specialist reference laboratories.

Outbreaks

It is noteworthy that most outbreaks have been traced to professional catering services or restaurants where cross-contamination has occurred between raw meat and cooked meat, or where foods have been undercooked. Again, the measures which caterers need to take to control other *Salmonella* spp. will readily control *S.* Typhimurium DT104.

Conclusions and implications for the future

From our present knowledge, the measures required to prevent foodborne illness by “ordinary” *Salmonella* spp. should be adequate to control foodborne illness caused by *S.* Typhimurium DT104. All processes in factories and catering operations must be based on good practices and HACCP. The long-term objective must be to eradicate *S.* Typhimurium DT104 and other drug-resistant microorganisms from the whole food chain. This will require a co-ordinated and concerted effort by governments and farming communities throughout the world.

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