Summary ESBL-Attribution-analysis (ESBLAT)

Searching for the sources of antimicrobial resistance in humans

Project number Topsector TKI-AF 12067

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ESBL Attribution Analysis

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ESBLAT Partners

One Health for Food (1H4F) Partners

ESBL attribution analysis

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Public partners

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Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht (IRAS)
National Institute for Public Health and the Environment, Bilthoven (RIVM)
Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, Utrecht (I&I)
University Medical Centre, Utrecht (UMCU)
Animal Health Service, Deventer (GD)
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Private partners

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Experts interviewed

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Introduction

What's the problem?

Since the turn of the century, ESBLs occurred increasingly in humans and animals. This is largely due to the use of antibiotics, but also increased mobility, trade of live animals and contamination through the environment contribute. That is of concern since ESBLs can degrade antibiotics that are important for humans and animals such as penicillins and cephalosporins, which may lose their efficacy. The extent to which livestock farming, the food chain and the environment contribute to carriage and infection in humans is unknown.

What are ESBLs?

Extended Spectrum Beta-Lactamases (ESBLs) are enzymes produced by bacteria that inactivate antibiotics that belong to the beta-lactam group. ESBLs inactivate ampicillin, amoxicillin and all cephalosporins. These antibiotics are very important for the treatment of infections in humans and animals. Bacteria that produce ESBLs are resistant bacteria. By inactivating beta-lactams they make these antibiotics ineffective for treating infections they cause.
There are many types of ESBLs that belong to an increasing number of groups, whose names always consist of a code with a number behind it. The most common ESBLs include the groups TEM, SHV and CTX-M. A common variant of the ESBLs is the CMY group (belonging to the plasmid transferable AmpC group). The number indicates the genetic variant within a group. Very common variants are CTX-M-1, 9, 14 and 15, TEM-20 and 52, SHV-2 and 12 and CMY-2.

Where do ESBLs occur?

The frequency of occurrence of ESBLs has increased in humans and animals since the year 2000. In humans this is largely caused by a worldwide (pandemic) distribution of CTX-M ESBLs (especially CTX-M-15) that are transmitted between bacteria via plasmids (see paragraph “How are ESBLs spread”). These plasmids have transferred into a successful human variant of *Escherichia coli*, namely ST131. This *E. coli* variant is mostly spread via human to human contact. In farm animals other ESBL variants are often found, of which CTX-M-1, TEM-52, CMY-2, SHV-12, CTX-M-14 and SHV-2 can be mentioned in order of the frequency of occurrence. These ESBLs are found in the faeces of all animal species usually without causing disease. In addition, poultry meat is most often contaminated, while ESBLs are found much less frequently in meat from other livestock species is. The ESBL enzyme predominantly found in humans, CTX-M-15, can incidentally be found in farm animals.

How are ESBLs spread?

The spread of ESBLs is extremely complex. This is because ESBL genes are transmissible within bacteria from chromosomal to plasmidic DNA. Plasmids are extra-chromosomal DNA structures that can multiply within a bacterium and can actively be transferred to other bacteria, independently of bacterial cell division, through a mechanism termed conjugation. Conjugation is most efficient between bacteria of the same species, for instance from one *E. coli* to another *E. coli*. However, it can also occur between bacteria that are less closely related, for example from *E. coli* to *Salmonella* or *Klebsiella*. Conjugation occurs in places where many bacteria are in close contact with each other such as the gastrointestinal tract of humans or animals, and possibly also in manure pits or sewers. Therefore, there are virtually no limits to the potential of ESBLs to spread.
Transmission of ESBLs within animals and between animals and humans can occur via bacteria whose ESBL-carrying plasmids can transfer to animal or human bacteria, including pathogens. The contribution of specific bacterial variants adapted to humans, such as *E. coli* ST131, appears to be less important as a source for animals. This means that to study the spread of ESBLs it is necessary to properly characterize both ESBL genes and the plasmids carrying them. Presence or absence of genes and/or plasmids can then be used to estimate the contribution of a given source to occurrence of ESBLs in humans.

What determines the occurrence of ESBLs?

The occurrence of ESBLs in humans and animals is the result of a combination of factors. Human and veterinary antibiotic use is the most important factor. It results in a positive selection of ESBLs, stimulates gene/plasmid spread in a given reservoir and increases the likelihood of transmission via conjugation. The positive selection mainly concerns agents that specifically select for ESBLs, such as cephalosporins; other antibiotics, as fluoroquinolones and aminoglycosides can also select ESBLs indirectly via co-selection. This occurs both in humans and animals.

Distribution and transfer of ESBLs is determined by inadequate hygiene and infection control measures in both humans and animals; animal movements within and between farms also contribute to transfer in farm animals. ESBL transmission between animals and humans depends on different types of exposure, for example direct contact, contaminated food or dust particulates in the stable environment. The molecular characteristics of gene/plasmid/strain combinations are also of extreme importance in determining whether a successful exchange occurs and if a carrier status is achieved. All in all, this process is very complex and difficult to predict.
Reduction of antibiotic usage in animal husbandry has been an important target of the Dutch governmental policy since 2008, because there was a major discrepancy (that partly still exists) in the Netherlands between antibiotic usage in humans (very low) and animals (very high until a few years ago). Extensive measures were initiated for the registration and reduction of antibiotic usage within the animal production sector which led to almost 65% reduction in sales of antimicrobials for use in livestock from 2009 to 2016. As a consequence the prevalence of ESBLs in farm animals and animal products has decreased in most livestock species.

What is known about the relationship between ESBLs from humans and animals?
Recent research in the veterinary and human sectors in the Netherlands has shown that there is a genetic relationship between part of the ESBLs that cause clinical infections in humans and those of poultry and poultry meat origin. However, ESBLs isolated from poultry (and meat) are also found in other animal species and in the environment. This suggests that there might also be other sources for the transmission of ESBLs to humans, including humans themselves. It is also unclear whether and to what extent transmission routes other than meat (i.e. plant products, the environment, or direct contact) contribute to human exposure. The relative importance of other sources for ESBL exposure, such as hospitalisation and travelling to high prevalence countries, is also unknown. Depending on the level of detail at which genetic relationships are investigated, it was estimated that 10 to 30% of clinical ESBL-producing *E. coli* isolates can come from livestock farming (especially broilers). It cannot be excluded that this relationship is subject to variations in region, time and source.

Goals

This project had two objectives:

1. To determine the contribution of all reservoirs to ESBL carrier status and infection in humans.
2. To determine the transmission routes from these reservoirs to humans.

The potentially most important reservoirs beside humans are farm animals: chickens, pigs and cattle and their breeding pyramids (see Fig. 1). Important transmission routes are food of animal origin (mainly meat) and plant origin, as well as the environment (especially water and soil), where numerous interactions between reservoirs and exposure routes exist. The contribution of direct contact between companion animals, horses and their owners was also studied. ESBL multiplication as a result of contact between individuals and selective pressure by antibiotic use in human medicine also needs consideration. Introduction of genes, plasmids, and/or bacteria via humans, animals, food and surface water must also be taken into account together with gene transfer via conjugation within and between all these reservoirs.
In humans a distinction is made between the general population (carrier status), patients in general practice (urinary tract infections) and patients in hospitals. The assumption is that carriage in the general population can lead to infections of the urinary tract with resistant bacteria and, consequently, hospitalization of some of these patients. In addition to the inflow of bacteria from the general population an independent cycle takes place within the hospital. For example, *Klebsiella* survives better than *E. coli* in hospitals. This factor is important to keep in mind when considering reservoirs and transmission routes.
ESBLAT's goal was to map the contribution of animal reservoirs to the occurrence of ESBL carrier status in humans. In order to do this, information was gathered about the occurrence of ESBLs in humans and various farm animals such as cattle, poultry and pigs, as well as pets. Moreover, the extent of the contribution of certain transmission routes, such as animal products (i.e. meat) and the environment (i.e. surface water), to the exposure of the general human population was investigated. This required information on ESBL types, ESBL prevalence (number of positive samples per reservoir) and the concentrations of ESBL-producing bacteria per reservoir. The objectives were reached by epidemiological analysis of the collected data, quantitative microbiological risk analyses (QMRAs) and a first attempt to mathematically model attribution.

Results

Studies in humans, animals and in the environment
To be able to take adequate measures in the future, the ESBLAT consortium investigated the contribution of animal, meat and the environment to the ESBL carrier status in humans (figure 1). In addition, the extent to which different transmission routes, such as meat consumption and swimming in surface water, contribute to the exposure of the general population was also examined. For this purpose, information was gathered about the presence of ESBLs in various farm animals such as cows, chickens and pigs, and also pets and wild birds. ESBL carriage in humans has been studied in people in the general population and livestock farmers. Moreover, in patients in hospitals and GP practices the proportion of ESBLs in *E. coli* infections was studied,

The genetic information of the different types of ESBLs, the extent to which they occur and the number of ESBL-producing bacteria per reservoir were collected from 35 Dutch studies with more than 27,000 observations in the period between 2005-2015. These studies had been carried out before or were initiated in the context of the ESBLAT research project (figure 1).

ESBLs are everywhere
ESBLs occurred in all reservoirs studied, both human, animal and environment. The highest percentages of ESBLs were found in poultry, on poultry meat and in surface water (50 to 100%). This was around 5% for the general populations. In patient groups studied, the proportion of ESBLs in *E. coli* infection was also around 5%. A higher probability of being a carrier was found in people who, because of their profession, are more likely to be exposed to animal reservoirs, such as farmers and slaughterhouse staff.

All ESBL types are everywhere
By mapping the genetic similarity of ESBL species between reservoirs (similarity analysis), an image is created of the possible transmission routes between these reservoirs (figure 2). The larger the genetic agreement, the more plausible that actual transfer took place. A wide variety of ESBLs was found in all reservoirs and transmission routes studied. A striking finding is that all ESBL species also occur in all reservoirs, albeit to varying degrees.

Exchange between people makes the biggest contribution
ESBL-types from the general population and patients show large genetic similarities, while types from livestock (animals, meat) are substantially less similar to those of humans (figure 2). The differences found between ESBLs in humans and in livestock farming suggest that farmed animals, including poultry and poultry meat, make a relatively small contribution to ESBLs that occur in humans compared to the human contribution itself.

The fact that man himself is the main source of ESBLs is in line with the recently released ECDC / EFSA / EMA report, in which the use of cephalosporins in human health care as the main cause for the occurrence of ESBL-producing *E. coli* in the man was called.

***Figure 1**. Prevalence (%) of ESBL/AmpC producing E. coli in 22 reservoirs (with permission taken from Dorado-Garcia et al. 2017).*

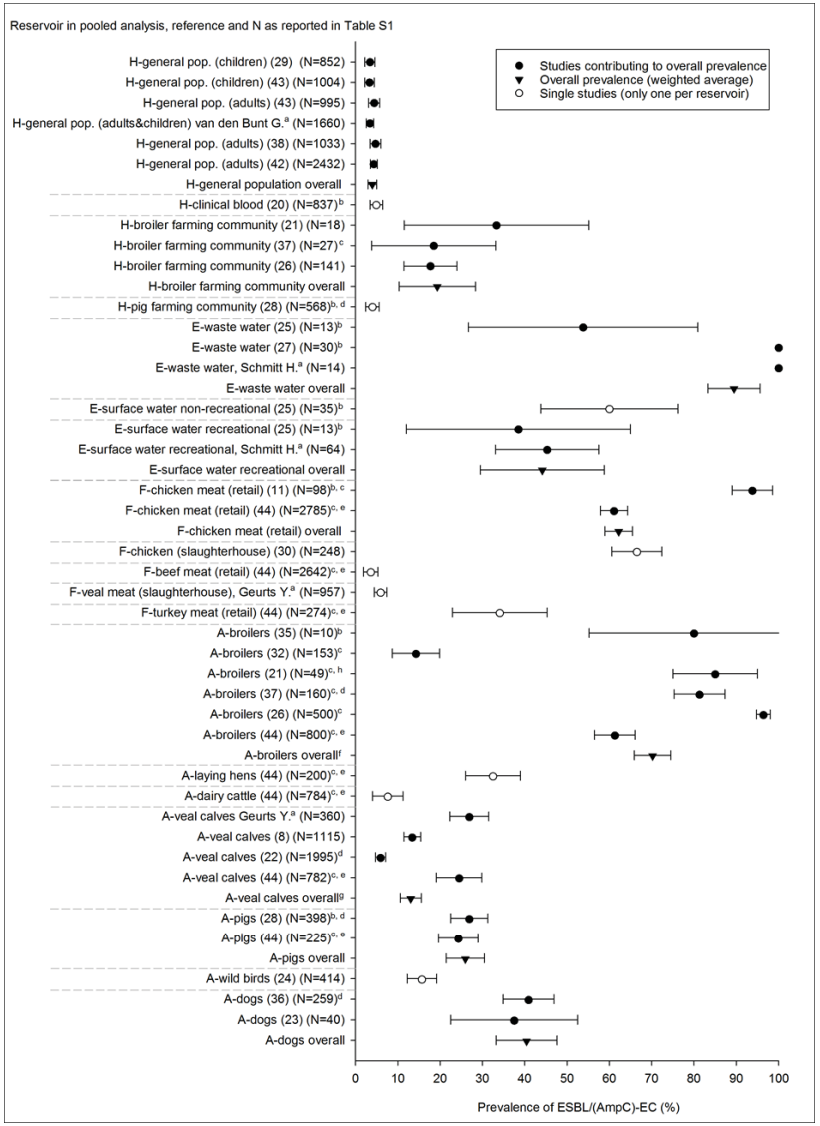
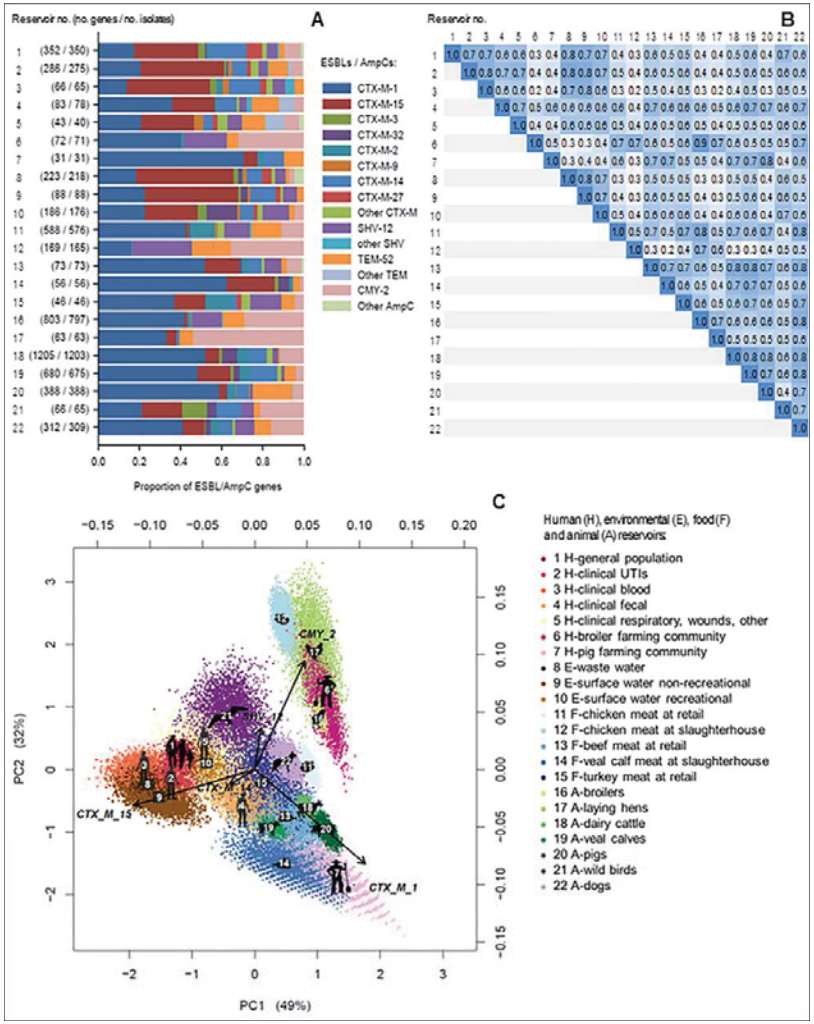


Figure 2. *A: Relative distribution of ESBL-genes identified in 22 reservoirs. B: Proportional similarity of ESBL-distributions between the 22 reservoirs (0 is no similarity and 1 is identical). C. Principal Component Analysis on bootstraps of distributions to visualize similarities and differences observed (copied from Dorado-Garcia, 2017 with permission).*



Livestock important source of ESBLs for farmers

Farmers are an exception. The distributions of ESBL species in farmers show a strong resemblance to those in their own livestock and differ from those of other population groups. This suggests that contact with livestock is the most likely transmission route.

ESBL contribution companion animals still unclear.

The ESBL types of dogs were similar to those of humans and livestock. This is probably partly explained by the intensive contact of the dog with his owner, but partly also by eating contaminated fresh or raw meat products and partly by exchanging ESBLs between dogs through intensive mutual contact. Whether direct contact with companion animals leads to a higher risk of ESBL carriers in humans has not been demonstrated within ESBLAT.

ESBL contribution through meat eating, swimming and livestock farming low

Based on swimming frequency, water absorption and water contamination, the exposure of swimmers is estimated. The same has been done for the exposure through consumption of pork, chicken and beef. To this end, data on the degree of contamination of meat and meat products are combined with information about the frequency of consumption.
The analyzes show that both swimmers and consumers are exposed to low concentrations of ESBLs. The exposure through consumption of meat, and in particular raw meat products, is higher than the exposure to ESBLs via swimming. At present, there are no epidemiological studies in the Netherlands that show that exposure through meat or swimming leads to an increased risk of carrier status in the general population or infections in patients.

Residents living in livestock-dense areas are exposed to ESBLs through the environment, for example by ESBLs in the air. Research among these residents showed that this exposure does not result in an increased chance of carrier status.

Mathematical model dissemination of ESBLs confirms low contribution animal and environment

The first exploratory attribution analysis, using existing models, supports previous studies that concluded that human ESBLs can only be attributed to animal sources and surface water to a limited extent. However, due to the many possible transmission routes and reservoirs, a quantitative analysis of the contribution to ESBL in humans is extremely complex (Figure 3). Even with the sizeable amount of new data obtained in the ESBLAT studies, questions remain. For example, about how long people and animals remain carriers of ESBL, what the relation between the level of exposure and the risk of carrier status (dose-effect relationships) and what the role of travelers returning with ESBLs is. Nor does the analysis provide a complete and insufficient quantitative picture of the transmission routes of different reservoirs to humans (direct contact, food, environment).

Human, animal and environment: one-health

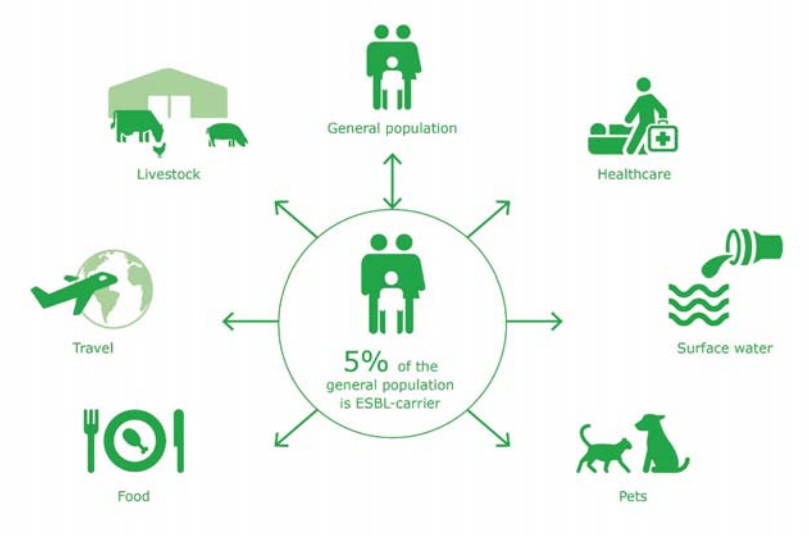
The omnipresence of ESBLs in humans, animals and the environment makes the detection of sources and transmission routes a typical *one-health* problem, which requires an interdisciplinary approach. The research within the ESBLAT consortium therefore involved a unique collaboration between experts in human health care and health research, veterinary medicine and environmental sciences. This cooperation was of great importance to be able to investigate and quantify the human exposure and health risks by humans, animals and the environment. This has led to important new insights into the presence and spread of ESBLs, as well as open questions. Given the dynamic nature of this problem, continuous attention in all areas therefore remains desirable.

Continuous ESBL monitoring is crucial

Man himself seems for the time being the most important source of ESBLs that cause infections in patients. Infection control and responsible use of antibiotics (*antibiotic stewardship*) in health care and veterinary medicine therefore remain important to prevent the spread of ESBLs.

Although the direct contribution from the food chain and surface water seems to be small at the moment, these are very large reservoirs that will always remain a source of exposure and dissemination. There is therefore a great need for additional longitudinal studies in a number of reservoirs to further improve the calculation models and to identify the contribution of sources. Monitoring of ESBLs in the different reservoirs is therefore crucial to recognize changes in dynamics in time.

Figure 3. *Source attribution model in which humans are included as source for other humans.*



Reference

Dorado-García A. et al, *Molecular relatedness of ESBL/AmpC-producing Escherichia coli from humans, animals, food and the environment: a pooled analysis*. J Antimicrob Chemother. **2017** Nov 18. doi: 10.1093/jac/dkx397.