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Associations between host characteristics and antimicrobial resistance of *Salmonella* Typhimurium

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SUMMARY

A collection of *Salmonella* Typhimurium isolates obtained from sporadic salmonellosis cases in humans from Lower Saxony, Germany between June 2008 and May 2010 was used to perform an exploratory risk-factor analysis on antimicrobial resistance (AMR) using comprehensive host information on sociodemographic attributes, medical history, food habits and animal contact. Multivariate resistance profiles of minimum inhibitory concentrations for 13 antimicrobial agents were analysed using a non-parametric approach with multifactorial models adjusted for phage types. Statistically significant associations were observed for consumption of antimicrobial agents, region type and three factors on egg-purchasing behaviour, indicating that besides antimicrobial use the proximity to other community members, health consciousness and other lifestyle-related attributes may play a role in the dissemination of resistances. Furthermore, a statistically significant increase in AMR from the first study year to the second year was observed.

Key words: Antibiotic resistance, environment–resistance relationship, epidemiological study, multivariate analysis, risk factor analysis.

INTRODUCTION

Antimicrobial use is seen to be the key reason for effective proliferation of resistant bacteria [1]. Nonetheless, showing the association between antimicrobial use of hosts and antimicrobial resistance (AMR) of strains they carried at the population level can be complicated. There are studies which were able to show this association based on individual patient data [e.g. 2, 3] and on surveillance data [4]. However, other studies observed no association [5, 6]. Several studies even showed the presence of resistant strains in humans or animals not using antimicrobials [6, 7]. This demonstrates that the dissemination of AMR is very complex and many factors may play a role.

Zoonotic bacteria like *Salmonella* are of special concern in monitoring programmes because of EU Zoonoses Directive 2003/99/EC [8]. Due to the use of antimicrobial agents in animals and humans, they may be subject to variable selective pressures [9]. In each host, various AMR genes may be acquired by horizontal transfer between strains of the same or different bacteria species [9, 10]. Underlining this, a recent study showed differences in resistance profiles

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of *Salmonella* Typhimurium DT104 occurring in animals and humans [11].

Concerning the spread of AMR between different individuals, it is assumed that resistance genes are transferred via the same routes as bacterial strains [10]. As meat is the main source for *S.* Typhimurium infection, indirect transmission of resistance genes between farm animals and humans via food is probable [12]. This assumption is supported by the association between raw meat handling and AMR found in *Escherichia coli* [13]. Transmission via direct contact of humans with animals is also possible, this having been shown for food animals for transmission of resistant *S.* Typhimurium [14] and for companion animals for transmission of resistant *Staphylococcus intermedius* [15]. Regarding human populations only, person-to-person spread was shown in studies regarding persons from same households or from the same communities [16, 17]. Sociodemographic attributes may reflect AMR patterns, e.g. proximity to other community members [6, 18], and contact with healthcare services [19] or daycare centres [20] were described as risk factors for AMR.

Another important point regarding resistance dissemination is that different resistance genes may be genetically linked, e.g. located together on a plasmid, and thus could be transferred together to other bacterial strains [10]. This increases the occurrence of resistance genes even if the particular antimicrobial was not used, which hampers the analysis of an association between resistance against a single antimicrobial and the use of that particular antimicrobial. Phenotypic susceptibility data are typically analysed without information on the presence of resistance genes or mobile genetic elements. Therefore, to avoid biased results and misinterpretation, it is beneficial to regard the whole resistance profile as outcome and include correlations between resistance properties by performing a multivariate analysis.

In this study, we conducted an explorative risk factor analysis for AMR of *S.* Typhimurium isolates collected within a case-control study of sporadic salmonellosis cases in humans from Lower Saxony, Germany [21, 22]. Individual patient data were available for sociodemographic factors, medical history, animal contact and food habits. Therefore, a detailed description of host behaviour covering the time before infection was given and associations between these factors and AMR were analysed. Investigations on linkages between resistance properties have been published for a part of the study data [23]. These confirm a high level of association between resistance properties within the test population and support the microbiological observation that resistance genes are often genetically linked and/or occur together. Therefore, in this study, entire resistance profiles were investigated as multivariate outcome in the risk-factor models using a non-parametric approach, performing between-group comparisons by permutation tests. Calculations were based on minimum inhibitory concentration (MIC) values.

MATERIALS AND METHODS

Study design

Case data were available from a case-control study on sporadic *Salmonella enterica* infections in humans in Lower Saxony, Germany, conducted between June 2008 and May 2010 by the State Health Department of Lower Saxony (Niedersächsisches Landesgesundheitsamt; NLGA) [21, 22]. Notified sporadic cases of salmonellosis that were not part of apparent local outbreaks were recruited and standardized questionnaires were completed via telephone interviews conducted by trained interviewers from NLGA and local public health departments of the districts. *Salmonella* isolates were made available through cooperating sentinel laboratories and were sent to the German National Reference Centre for *Salmonella* at the Robert Koch-Institute, Wernigerode, where the serotype, phage type and antimicrobial susceptibility profile were determined. As isolates with different serotypes show different resistance distributions, in this study we focused on *S.* Typhimurium; this was, with 52% ($n = 383$), the most frequent serotype in the sampling period.

Host data

The questionnaires comprised questions concerning risk factors for salmonellosis, which were divided into four groups: general patient information, medical history, animal-related factors, and food-related factors. Analysing categorical variables which had a minimum of eight observations in each category, a total of 63 factors were investigated which are listed in Table 1.

Phenotypic data

Serotyping of *S.* Typhimurium was conducted according to the White–Kauffmann–LeMinor scheme by slide agglutination with O- and H-antigen-specific sera [24]. Phage typing of *S.* Typhimurium isolates was performed in compliance with previous work [25, 26].

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Table 1. *List of host factors analysed to investigate the association with antimicrobial resistance*

General patient information		
Sex[a]	Age[b]	Region[c]
Study year[d]	Foreign travel[e,f]	
Medical history[e,g]		
Pre-existing condition		
Infection	Cancer	Immune deficiency
Intake of		
Antimicrobial agents	Gastric acidity inhibitors	Corticosteroids
Other drugs		
Animal-related factors[e,f]		
Job-related animal contact of a member of the household		
Husbandry of		
Dogs	Cats	Rodents
Birds	Cows	Horses
Contact with		
Dogs	Cats	Rodents
Reptiles	Birds	Cows
Pigs	Horses	
Food-related factors[e,f]		
Specific diet	Meals outside the household	Barbecue
Consumption of		
Egg (in any form)	Undercooked egg	Pasteurized egg
Raw egg	Fish	Meat (in any form)
Raw ground pork	Raw or medium beef	Chicken
Turkey	Duck or goose	Sausage
Uncooked pork sausage	Raw milk	Raw vegetables or fruits
Raw tomatoes	Raw garlic	Salty snacks
Peanuts	Chocolate	Sweets
Herbal tea	Spices (in any form)	Black pepper
Mixed spices	Liquid spice	Non-heated fresh herbs
Non-heated dried herbs		
Buying behaviour		
Direct producer egg delivery	Purchasing barn eggs	Purchasing free-range eggs
Meat from the market	Meat from the meat counter	

Factors with significant association with antimicrobial resistance are underlined (see Table 4).
[a] Female, male.
[b] 0 to $\leqslant$2 years, 2 to $\leqslant$14 years, 14 years.
[c] Conurbation area, urbanized area, rural area (according to a region type classification in [37]).
[d] June 2008 to May 2009, June 2009 to May 2010.
[e] Yes, no.
[f] Concerning 3 days before onset of disease.
[g] Concerning 4 weeks before onset of disease.

Antimicrobial susceptibility tests were conducted by broth microdilution according to document 58940-8 of the German Institute for Standardisation (Deutsches Institut für Normung; DIN) [27] obtaining MIC values for 13 antimicrobial agents (Table 2). *E. coli* ATCC® 25922 served as quality control strain. For calculations with MIC values, we used the following conventions: if the lowest test concentration of

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Table 2. *Tested antimicrobial agents, dilution ranges, clinical breakpoints (μg/ml) and proportions of resistant strains for* Salmonella *Typhimurium isolates (n=383)*

Class of antimicrobial agent	Antimicrobial agent	Abbreviation	Test range (μg/ml)	Clinical breakpoint (μg/ml)		R, %
				S	R	
β-lactams (penicillins)	Ampicillin	AMP	1–16	⩽2	⩾16	78·3
β-lactams (penicillins) + β-lactamase inhibitor	Mezlocillin + sulbactam	MSU	2–32	⩽4	⩾32	9·9
β-lactams (cephalosporins, 2nd generation)	Cefotiam	CTM	0·5–8	⩽4	⩾16	0
β-lactams (cephalosporins, 3rd generation)	Cefotaxime	CTX	1–16	⩽2	⩾16	0
Quinolones	Nalidixic acid	NAL	4–32	⩽16	⩾32	4·7
Fluoroquinolones	Ciprofloxacin	CIP	0·0625–64	⩽1	⩾4	0
Aminoglycosides	Streptomycin	STR	4–64	⩽8	⩾32	77·6
	Kanamycin	KAN	2–32	⩽16	⩾64	8·6
	Gentamicin	GEN	0·5–8	⩽1	⩾8	0·3
	Amikacin	AMK	2–32	⩽4	⩾32	0
Tetracyclines	Oxytetracycline	OTE	0·5–8	⩽1	⩾8	77·6
Potentiated sulfonamides	Trimethoprim + sulfamethoxazole	SXT	4–128	⩽16	⩾128	15·7
Phenicols	Chloramphenicol	CMP	4–32	⩽8	⩾16	14·6

S, Susceptible; R, resistant.

an antimicrobial agent already inhibited growth, the corresponding MIC value was recorded as being equal to this concentration. If the highest test concentration of an antimicrobial agent did not inhibit growth, the corresponding MIC value was recorded as the next concentration in a twofold dilution series.

Furthermore, the isolates were categorized as resistant (R) and non-resistant (NR, summarizing susceptible and intermediate isolates) using clinical breakpoints recommended in DIN 58940-4 [27]. If DIN breakpoints were not available, breakpoints given in document M100-S20 [28] of the Clinical Laboratory and Standards Institute (CLSI) were used. This was done for nalidixic acid and kanamycin. As no internationally accepted breakpoints exist for streptomycin we used the breakpoints accepted by the Robert Koch-Institute in their long-term *Salmonella* monitoring programmes. The breakpoints used are listed in Table 2. Isolates were classified as multi-resistant when they exhibited resistance to at least three antimicrobial agents of different classes/subclasses [29].

Statistical models

Risk-factor analyses with multivariate MIC profiles as outcome were conducted using distance-based permutation tests suggested by Anderson [30] and McArdle & Anderson [31]. This approach is non-parametric and appropriate for unbalanced and multifactorial designs. For this case, the $n \times p$ data matrix **Y**, containing the 2-log-transformed MIC values on n isolates for p antimicrobial agents, was transferred into the $n \times n$ distance matrix **D**, whose elements d_{ij} reflect the distance between isolate i and isolate j with respect to the p 2-log-transformed MIC values (for distance calculation see below). Summing up the distances between all possible pairs of isolates gives an estimator for total variability. The total variability can be partitioned into the variability between groups and the variability within groups by estimating the variance components based on distances only. The test statistic for conducting between-group comparisons of MIC profiles is then the ratio of both variance components, which follows the same philosophy as in analysis of variance (ANOVA) for univariate data. In the case of a multifactorial model the total variation is partitioned into several variance components, again directly from the distance matrix, and appropriate test statistics are formalized. In the case of only one outcome variable ($p=1$) and calculating distances as Euclidean distance, the test statistic for testing one risk factor is equal to the common F statistic in a one-way ANOVA. However, for this approach any metric or non-metric distance measure can be chosen,

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Table 3. *Resistance characteristics for* Salmonella *Typhimurium subtypes analysing antimicrobial resistance against 13 antimicrobial agents*

Subtype	*n*	MNR	MR, %	NR, %	Most frequent RP, %	No. of RP
All isolates	383	2·9	74·2	14·4	AMP+STR+OTE (42·8)	37
DT104 biphasic	32	4·0	87·5	0·0	AMP+STR+OTE+CMP (43·8)	6
DT120 monophasic	24	2·9	75·0	12·5	AMP+STR+OTE (54·2)	9
DT120 biphasic	67	2·4	56·7	34·3	nr (34·3)	17
DT193 monophasic	137	3·1	94·9	0·7	AMP+STR+OTE (83·9)	12
DT193 biphasic	43	3·7	83·7	0·0	AMP+STR+OTE (20·9)	15
Others	80	2·0	42·5	35·0	nr (35·0)	20

MNR, Mean number of resistances; MR, multi-resistant isolates; NR, fully non-resistant pattern; RP, resistance profile.

and it can be applied to variables with any measurement levels [30, 31]. Appropriate P values can be obtained using permutation tests.

Distances between MIC profiles were calculated as follows: as tested concentration steps for generating MIC values were limited, observed MIC distributions occurred as truncated and a large number of ties were possible. Therefore, the level of measurement for MIC values was understood as ordinal and the Manhattan distance was used. The distance d_{ij} between isolate i and isolate j, with $i, j = 1, \ldots, n$ and $i \neq j$, was calculated by

$$d_{ij} = \sum_{k=1}^{p} \frac{|y_{ik} - y_{jk}|}{c_k},$$

where y_{ik} and y_{jk} denote the 2-log MIC for antimicrobial agent k, $k = 1, \ldots, p$ of the ith and jth isolate, and c_k is the number of tested concentration steps for antimicrobial agent k. Scaling the distances by the particular test ranges, leads to a maximum distance of 1 for each antimicrobial agent and to a maximum distance of p for d_{ij}.

For each host characteristic (see Table 1) a model was generated to investigate the association with the multivariate MIC profiles. As resistance distributions depend on phage type, this was considered as a confounder variable in each model with six categories of isolates: DT104, DT120 monophasic, DT120 biphasic, DT193 monophasic, DT193 biphasic, and others (see Table 3). Therefore, each host characteristic was examined within a two-factor model involving phage type, the host characteristic and an interaction term as fixed factors. Analyses were conducted with R version 2.15.2 [32] with function adonis, package vegan version 2.0–4 [33]. For all tests a subset of 9999 permutations was used. In this study P values $\leqslant 0{\cdot}05$ were interpreted as statistically significant. In cases where two-way interactions were not meaningful (less than three observations per group) or did not appear to be statistically significant, models with main effects only were investigated. If there was a statistically significant result for testing a factor or an interaction with three or more groups, we conducted pairwise comparisons between groups. As this study is of an exploratory nature, no α-adjustment to account for multiple hypotheses testing was applied. For interpreting significant differences between groups, mean number of resistances (MNR) were calculated to quantify the degree of resistance per group. To visualize group differences in MIC distributions MIC_{10}, MIC_{50} and MIC_{90} are displayed, which are the 10%, 50% and 90% distribution percentiles [29].

For evaluating MIC profile-based results, each model was recalculated replacing the MIC profile with the binary-coded resistance profile based on the information resistant or non-resistant. For this, the distance-based permutation tests were performed using the simple matching coefficient to obtain distances between the binary-coded profiles.

RESULTS

During the whole study period 867 notified sporadic cases with identified serovar Typhimurium were recorded with a median age of 13 years and 53·9% male participants [22]. Of these, 540 cases were recorded in the first study year (median age 13 years, 56·9% males) and 327 cases were recorded in the second year (median age 11 years, 48·9% males). Phage typing and susceptibility tests were conducted for 383 of these isolates (first year, 250 isolates; second year, 133 isolates). In this study population the median age was 12 years (first year, 12 years; second

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Table 4. *Descriptive information on host-specific factors with significant results ($P \leqslant 0{\cdot}05$) in two-factorial models with adjustment for phage types analysing the association with antimicrobial resistance for* Salmonella *Typhimurium isolates ($n = 383$)*

	MNR of group 1, MNR of group 2[a] (*P* value)						
		Phage type subgroup[c]					
Factor	All[b]	DT104	DT120 monophasic	DT120 biphasic	DT193 monophasic	DT193 biphasic	Other
General patient information							
Study year (1, 250; 2, 133)	2·7, 3·1 (0·043)[d]						
Region (CA 86, UA 219, RA 78)[f]	(<0·001)[e]						
Region (CA *vs.* UA)		3·7, 4·1 (0·392)	3·2, 2·8 (0·654)	5·5, 2·0 (<0·001)	3·3, 3·0 (0·038)	4·3, 3·4 (0·105)	2·4, 2·2 (0·503)
Region (CA *vs.* RA)		3·7, 4·3 (0·382)	3·2, 2·9 (0·562)	5·5, 2·3 (0·001)	3·3, 3·0 (0·104)	4·3, 3·9 (0·468)	2·4, 1·2 (0·085)
Region (UA *vs.* RA)		4·1, 4·3 (0·141)	2·8, 2·9 (0·630)	2·0, 2·3 (0·667)	3·0, 3·0 (0·594)	3·4, 3·9 (0·191)	2·2, 1·2 (0·165)
Medical history (previous 4 weeks)							
Antimicrobial intake (yes 42, no 341)	(0·008)[e]	4·0, 4·0 (0·802)	3·7, 2·8 (0·356)	4·6, 2·2 (0·002)	3·3, 3·1 (0·083)	3·8, 3·6 (0·748)	1·9, 2·0 (0·813)
Food-related factors (previous 3 days)							
Direct egg delivery from producer (yes 78, no 305)	(0·005)[e]	3·8, 4·0 (0·679)	2·3, 3·0 (0·253)	1·1, 2·8 (0·011)	3·1, 3·1 (0·450)	4·1, 3·5 (0·753)	2·4, 1·9 (0·218)
Barn eggs (yes 74, no 309)	3·3, 2·8 (0·014)[d]						
Free-range eggs (yes 126, no 257)	(0·023)[e]	3·6, 4·3 (0·268)	2·9, 2·9 (0·821)	1·5, 2·8 (0·015)	3·1, 3·1 (0·823)	3·7, 3·6 (0·787)	2·3, 1·9 (0·363)

[a] MNR, Mean number of resistances.
[b] In the case of significant phage-type interaction only the *P* value is given and MNRs are given at the phage-type level.
[c] Information is given at phage-type level, if the phage-type interaction was significant.
[d] Significant main factor, interaction was not significant.
[e] Significant phage-type interaction.
[f] CA, Conurbation area; UA, urbanized area; RA, rural area.

year, 12 years) and the proportion of males was 53·0% (first year, 56·8%; second year, 45·9%).

Frequencies of observed phage types are given in Table 3. Testing the association between these phage-type categories and the multivariate MIC profiles in a one-factor model resulted in a global P value of <0·001. Each pairwise comparison showed statistically significant differences except for the comparison between DT120 monophasic and DT120 biphasic isolates ($P = 0·155$) as well as the comparison between DT120 biphasic isolates and the group of other phage-type isolates ($P = 0·636$).

Group MNRs and P values are listed in Table 4 for host factors, which showed statistically significant associations with multivariate MIC profiles in the two-way models. Corresponding MIC distributions are shown in Figure 1.

Concerning variables on general patient information, differences between study years and region types were found to be statistically significant. Comparing the MIC distributions and the MNRs, the second study year tended to have higher MIC values and higher numbers of resistances than the first year independent of phage type. Region type was statistically significant for phage-type DT120 biphasic isolates, comparing conurbation with urbanized areas and conurbation with rural areas and for phage-type DT193 monophasic isolates, comparing conurbation with urbanized areas. In these cases isolates of patients living in conurbation areas tended to have higher MIC values than the other two groups.

For medical history, antimicrobial consumption had a statistically significant association with AMR for phage-type DT120 biphasic isolates, where the group with consumption showed an increased MNR and higher MIC values. For other subgroups no significant associations were observed.

While no animal-related factor was statistically significant, three food-related factors were detected, all referring to egg-purchasing behaviour. The association between AMR and the factors direct producer delivery and purchasing free-range eggs depended on phage type. For both, DT120 biphasic isolates of patients with that particular behaviour had lower MIC values and lower MNRs than those of patients without the behaviour. The other phage-type subgroups were not significant. Purchasing barn eggs was associated with MIC values independent of phage type. Higher values and higher numbers of resistances were found in isolates of patients that purchased barn eggs.

DISCUSSION

The objective of this study was to identify and assess the importance of factors associated with AMR in *S*. Typhimurium isolates from sporadic salmonellosis cases in humans from Lower Saxony, Germany. Lower Saxony is a federal state where agriculture and livestock farming are of major importance. It is located in north-west Germany and the total population is 7·9 million with a population density of 166·5 residents/km^2 [34].

Testing the association between phage type and AMR resulted in a highly statistically significant P value. Nearly all DT104 and DT193 monophasic and biphasic isolates were multi-resistant (Table 3). On the other hand, for DT120 biphasic isolates and the group of other phage types the most common resistance profile was the fully susceptible pattern. These phage-type-specific resistance distributions imply that it is necessary to analyse resistance data at the phage-type level, not only on serotype level as is commonly done. In cases where phage types are distributed differently in the risk factors of interest, the estimated effect will be biased. In this study, we included phage type as a confounder in each model and tested for interactions instead of conducting a stratified analysis. In cases of no significant interaction we were able to interpret the results at the serotype level, thereby gaining a higher sample size and more confidence in the results.

For interpreting statistically significant host characteristics we assumed that persons with risk factors have an increased probability of having a higher frequency of resistant bacteria in their faecal flora and/or that the *S*. Typhimurium isolates transmitted to those persons have a higher probability of having increased MIC values.

Regarding antimicrobial use, it was known whether a person in the study population had taken any antimicrobials during the previous 4 weeks before onset of the disease, but no further specifications concerning agent or duration of use were available. Antimicrobial use was associated with increased AMR in DT120 biphasic isolates.

In the 2-year study period higher MIC values were observed in the second year. This trend was observed continually between 1999 and 2008 in German *Salmonella* monitoring [35]. Although absolute numbers of prescriptions in Germany remained almost constant from 1991 to 2011, the proportion of reserve antimicrobials, e.g. fluoroquinolones and

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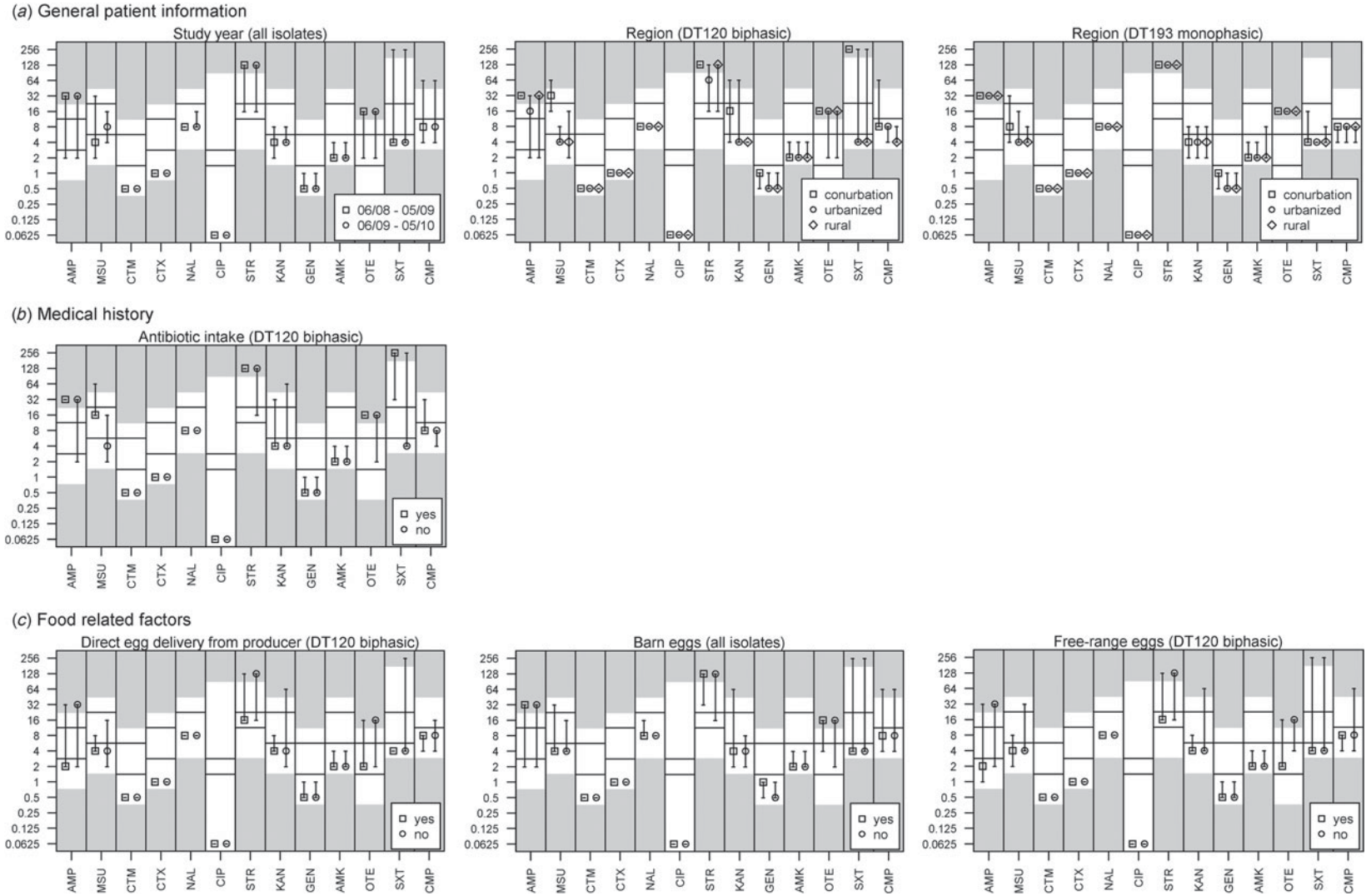

Fig. 1. Comparison of MIC distributions between groups of significant host characteristics. (In cases of significant interactions only significant phage-type subgroups are presented. The symbols represent locations of MIC_{50}. The vertical lines represent the range between MIC_{10} and MIC_{90} in each group. Horizontal lines represent the clinical breakpoints used. The concentration ranges tested are those contained in the white area. Values above this range are due to isolates which were not inhibited by the largest tested concentration, values corresponding to the lowest concentration are due to isolates which were already inhibited by the smallest tested concentration.)

cephalosporins, increased continuously [36]. In agreement with this finding, in our study the MIC_{90} increased for nalidixic acid in the second year.

Region type was the factor with the lowest *P* value when testing for the association with AMR. Increased MIC values and number of resistances were observed for conurbation areas for DT120 biphasic and DT193 monophasic isolates. The region categories were defined based on structural characteristics for settlements combining aspects of population density and accessibility of centres [37]. A descriptive comparison between region type and district-based ambulant antimicrobial prescription data for children (aged 0–17 years) for 2010 [38] showed no clear association. For adults no German data at district level have been published. Assuming that prescriptions for adults are correlated with those for children, we do not suppose that antimicrobial use may explain the observed region association. Effects on AMR were suggested for contact with other community members in isolated populations in Nepal [6] and for population density comparing populations of three cities in Canada, Greece and The Netherlands [18]. These factors are associated with these region types by definition and may play a role in this study, too. In general, assuming that different region types represent different lifestyles of persons, many factors may be associated with it and this may be the real risk factor behind region type for AMR. The only conclusion we can draw is that the overall exposure to AMR was estimated to be larger for persons living in conurbation areas.

No animal-related factor was identified as a risk factor for AMR. The smallest *P* value was observed for having contact with dogs, with $P=0{\cdot}090$ for testing the interaction with phage type. It should be noted that in the study population only a few individuals had contact with cattle, pigs, horses or reptiles. Therefore, phage-type adjustment was not possible and results for these species are not very reliable.

The only statistically significant results for food-related factors were observed for the three factors concerning egg-purchasing behaviour, where purchasing barn eggs was associated with higher MIC values and increased number of resistances while free-range eggs and direct egg delivery were associated with lower MIC values and decreased number of resistances. As eggs are not a typical infection source for *S.* Typhimurium, the spread of resistance while handling or consuming eggs was not assumed to be important here. A study on Canadian egg consumers found that health-conscious consumers were willing to pay more for eggs when they had special attributes, e.g. free-range or Omega-3 eggs [39]. As the proportion of sold cage eggs is very low and decreasing further, barn eggs can be seen as the cheapest egg class available in German food retailing [40]. Purchasing free-range eggs or purchasing eggs directly from the producer would then, assuming the same association here, reflect a greater health consciousness. As these considerations are speculative, more specific data are needed for further investigations.

The multivariate non-parametric model approach used here was first introduced for applications in ecology [30, 31]. As calculations are based on distances only (and any distance measure can be chosen), this method can be applied where in principle a multivariate analysis of variance (MANOVA) design is available but the outcome variables do not fulfil the assumption of being normally distributed. In this study, we assessed the MIC values for 13 antimicrobial agents as outcome. These are ordinal and the Manhattan distance was used. Alternatively, resistance profiles using the classified information 'resistant' and 'non-resistant' may be analysed with this approach using, e.g. the simple matching coefficient to measure distances. Analysing the associations between host characteristics and AMR based on these binary-coded resistance profiles with adjustment for phage type resulted in identifying the same statistically significant factors, interactions and pairwise comparisons with similar *P* values as in the analysis of MIC profiles except for study year, for which testing the main factor resulted in $P=0{\cdot}265$. This shows that variation in MIC profiles corresponded basically with variation in the binary-coded resistance profiles in this study. For study year, the group MNRs show slight differences (Table 4), but the MIC distributions in Figure 1 show that group differences in MIC_{10}, MIC_{50} or MIC_{90} values were observed below the resistance breakpoint, and cannot be reflected in the binary-coded profiles. Therefore, the advantage of analysing MIC values is that variation below and above the breakpoint is accounted for. For *Salmonella* and other species, changes concerning low resistance antimicrobials like ciprofloxacin or the cephalosporins are of special interest. For these, slight increases are relevant, even when values remain below the breakpoint.

Like MANOVA (and even univariate ANOVA) this distance-based approach is based on the assumption of homoscedasticity, which is that group variances have to be equal [31]. If this assumption

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does not hold, a factor may be declared significant due to differences in location or differences in dispersion. In our opinion, for susceptibility data analysis any kind of differences associated with a special host characteristic may be interesting for collecting information on the dissemination of resistance genes. Therefore, the test results are interesting in both a homoscedastic and a heteroscedastic situation, but for the interpretation a description of location and variation within groups has to be considered. This was done here by displaying the MIC_{50} and the range between MIC_{10} and MIC_{90} per group and antimicrobial agent.

Limitations of this study arose due to sample size. All results observed are based on two-way risk-factor models, screening for significant associations between single host characteristics (Table 1) and multivariate resistance profiles with adjustment for phage type of isolates. An adjustment for further covariates and analysing multifactorial models would have provided further insight into associations with AMR. In this study, it was not possible to do this as subgroups would have been too small for meaningful comparisons with adequate power. Furthermore, as this study was of an exploratory nature, no α-adjustment was made for multiple pairwise comparisons between groups. Therefore, this study should be considered for generating hypotheses and all results reported here need to be confirmed in future studies.

In summary, by analysing multivariate resistance profiles of MIC values we determined statistically significant phage-type adjusted associations with antimicrobial intake, study year, region type and three factors on egg-purchasing behaviour for *S*. Typhimurium isolates collected in Lower Saxony, Germany. We conclude that besides antimicrobial use other host characteristics such as proximity to other community members, health consciousness and other lifestyle-related factors may play an important role in the dissemination of resistance genes. As this study was of an exploratory nature, all observed results should be interpreted as initial evidence, and further research is needed for validation.

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DECLARATION OF INTEREST

None.

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