Salmonella in pigs and pork and their antimicrobial resistance - 10 years of surveillance in Germany

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Abstract
Salmonella from pigs and pork have been submitted to the National Reference Laboratory for the Analysis and Testing of Zoonoses (Salmonella) (NRL Salm) for a number of years. This study retrospectively analyses the data of Salmonella and their antimicrobial resistance generated between 2000 and 2009. A total of 4163 isolates from pigs and 1839 isolates from pork submitted to the NRL Salm were serotyped and tested for antimicrobial resistance using the broth microdilution method. Minimum inhibitory concentrations (MIC) were evaluated using epidemiological cut-off values as provided by EUCAST (www.eucast.org) at the time of interpretation (2010).

The majority of isolates from pigs and pork belonged to three serovars: S. Typhimurium (66 and 52%), monophasic variant of S. Typhimurium [1,4,[5],12:i:-] (11 and 10%) and S. Derby (7 and 10%). In both origins the number of S. Typhimurium decreased by roughly 50% while monophasic variant of S. Typhimurium increased from zero to 32 and 26%, respectively. The proportion of S. Derby varied between 5 and 12% in both origins.

Antimicrobial resistance in S. Typhimurium was high. In pigs there was a slight decrease in resistant isolates from 97 to 88% over the ten year period. In pork, the proportion of resistant isolates was lower but remained constant at about 80%. In the monophasic variant of S. Typhimurium susceptible isolates were rare (3%) and the majority (>80%) of isolates was resistant to streptomycin, ampicillin, sulfamethoxazole and tetracycline. In S. Derby, resistance was substantially lower (55%) compared to S. Typhimurium and the monophasic variant of S. Typhimurium. Resistant isolates to 3rd generation cephalosporins were rare (<1%) in pigs and absent in pork. However, in 2008 four isolates (0.8%) and in 2009 seven isolates (2%) indicated a potential emergence of resistance to this group of antimicrobials. Resistance to fluoroquinolones was constantly on a low level in pigs and pork (3 to 4%). Similarity of trends in serovar and resistance patterns of animal and food derived isolates supports the assumption of vertical transmission along the food chain.

Introduction
Resistance in zoonotic pathogens potentially hampers treatment of severe human infections. Food is an important source for Salmonella causing human infections. Moreover, Salmonella in food often originates from animals that harbour Salmonella or from cross contamination during slaughter and processing. It was the purpose of this study to investigate the development of antimicrobial resistance in Salmonella from pigs and pork over a 10 year period.

Material and Methods
Isolates had been submitted for diagnostic purposes to the NRL Salm between 2000 and 2009. At the NRL isolates were confirmed as Salmonella and serotyped. Antimicrobial resistance testing was carried out using the broth microdilution method according to CLSI-standards by the National Reference Laboratory for Antimicrobial Resistance. All isolates were evaluated using the same epidemiological cut-off values as provided by the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) at the time of analysis (2010). More details as well as test ranges can be obtained from the reports published by BfR (www.bfr.bund.de).

Results
A total of 4163 isolates from pigs and 1839 isolates from pork submitted to the NRL Salm were serotyped and tested for antimicrobial resistance. Figures 1 and 2 display the proportion of the most frequent Salmonella serovars based on the frequency observed in 2009 for pigs and pork, respectively. S. Typhimurium was the most frequently detected serovar in most years. However, its proportion decreased from more than 80% to less than 50% in pigs and from more than 60% to
below 40% in pork. Its share was partly replaced by the monophasic variant of Salmonella Typhimurium named S. 1,4,[5],12:i:- that increased from 0% in 2000 to 30% (pigs) and 25% (pork) in 2009. In contrast, the proportion of S. Derby was more or less constant over time.

In 2009, 81% of the Salmonella isolates from pigs were resistant to at least one class of antimicrobials and 75% to more than one. The majority of resistant isolates is linked to the serovar S. Typhimurium and his monophasic variant S. 1,4,[5],12:i:- that were resistant in 87.1% and 96.4% of isolates and multiresistant in 81.9% and 94.6%, respectively. The respective resistance rates for S. Derby were 40% and 35%. In contrast, most isolates (7/8) of S. Enteritidis were fully susceptible.
In 2009, S. Typhimurium was resistant to aminopenicillins, streptomycin, tetracycline and sulfamethoxazole (73-83%). Resistance to amphenicols (34-40%), trimethoprim (32%) and kanamycin (13%) was less frequent. Only 2 isolates were resistant to third generation cephalosporines and (fluoro-)quinolones.

The resistance pattern of the monophasic serovar S. 1,4,[5],12:i:- was similar to S. Typhimurium with a predominance of a fourfold resistance to ampicillin, streptomycin, sulfamethoxazole and tetracycline. Few isolates were resistant to cephalosporines (2/110, 1.8%) und ciprofloxacin (1 isolate). Resistance to amphenicole and trimethoprim was less frequent than in S. Typhimurium. In contrast, resistance to streptomycin, ampicillin, sulfamethoxazole and tetracycline was even higher than in S. Typhimurium.

S. Derby was less frequently resistant to all of the substances tested. None of the isolates was resistant to third generation cephalosporines or (fluoro-)quinolones.

Overall the proportion of resistant and multiresistant Salmonella isolates in pigs decreased slightly over the years (from 93 and 82% in 2000 to 81 and 75% in 2009). Resistance to amphenicole and (fluoro-)quinolones tended to decrease in S. Typhimurium (from 56% in 2000 to 35% in 2009). On the other hand, resistance to gentamicin and kanamycin slightly increased. Resistance to third generation cephalosporine was on a low level in all years but tended to increase between 2007 and 2009.

Resistance of S. Derby fluctuated substantially over the years (between 11 and 60%). This may in part be attributed to the limited number of isolates and variations in the submission pattern.

![Fig. 3: Resistance of S. Typhimurium from pigs and pork in Germany to selected antimicrobials (2000 – 2009)](image)

In pork, the proportion of resistant (67%) and multiresistant (52%) isolates was lower over the years compared to isolates from pigs (Figure 3). This also applied specifically for S. Typhimurium and S. 1,4,[5],12:i:-: However, the observed resistance pattern was similar, which is in line with the observed similarities in serovar patterns. There were some fluctuations, so that no clear trend could be observed for resistance and multiresistance. Interestingly, the changes in resistance observed in isolates from pigs were not observed in those from pork. The overall level of resistance to amphenicole and especially to (fluoro-)quinolones had been lower from the beginning and there was only a slight additional decrease concerning amphenicole.
Discussion and conclusions

There were great similarities between isolates from pigs and pork concerning the serovar and resistance patterns. This underlines that isolates in food often originate from primary production and cross contamination during slaughter and processing. Overall, isolates from food were less resistant than those from primary production. This difference was not fully attributable to a different serovar pattern because differences were also observed within the same serovars (S. Typhimurium and S. 1,4,[5],12:i:-). A potential factor could be that meat is more or less uniformly derived from one age group of pigs while isolates from pigs are derived from all age groups including piglets and weaners that might have a different degree of exposure to antimicrobials leading to the selection of more resistant strains. In addition, insufficient information was available on the origin of the meat (national production or imported products.

In isolates from pigs, the decrease in resistance is a positive development. The decrease in resistance to chloramphenicol can be attributed to a long term effect of the ban of this substance from animal production in Europe. The decrease in resistance to fluoroquinolones was less expected as fluoroquinolones are licensed for use in pigs, although not for oral medication. However, limited information is available on the amount of fluoroquinolones used in pig production in Germany, which underlines that consumption data are urgently needed.

The slight increase in isolates resistant to 3rd generation cephalosporines warrants attention, as 3rd and 4th generation cephalosporines are considered critically important for human medicine.

More data on the resistance of the isolates can be obtained from extensive reports published by BfR (www.bfr.bund.de).