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PLASMID PROFILES AS A MEANS OF DIFFERENTIATING *SALMONELLA* AND *CAMPYLOBACTER* ISOLATES FROM THE MEAT OF POULTRY

VICTORIA ATANASSOVA and CHRISTIAN RING

Veterinary University Hannover; Center for Food Science; Dep. of Food hygiene and -microbiology

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Introduction

The occurrence of bacteria (*Salmonella, Campylobacter, Staphylococcus aureus, Listeria monocytogenes* and *Clostridium botulinum*), as well as mycotoxin-producing fungi in food is a very topical problem in food hygiene. In poultry and in poultry meat, the occurrence of *Salmonella* has a main risk potential for consumers.

Contamination rates in poultry laying flocks for *S. enteriditis* or *S. typhimurium*, for example, seem to vary from 3% up to 50% in the member states of the European Union. The contamination of broilers with *S. enteritidis* may even be higher.

For diagnostics and epidemiological analysis of *Salmonella* and *Campylobacter*, classical methods of differentiation are almost always applied, including pre-enrichment, selective enrichment, isolation, as well as serological and biochemical confirmations. In contrast, molecular biological methods are novel means of typing isolates of bacteria. Among these are DNA-fingerprinting (RAPD-Mapping) or the use of DNA-probes, as well as PCR (polymerase chain reaction). Single techniques can be combined in typing systems. Such systems are well-suited for confirming details of the source and development of food poisoning cases. One such system which we have tested practically for typing *Salmonella* and *Campylobacter* isolates is the demonstration of plasmid profiles. This is described in the present study.

Materials and methods

Salmonella isolates

During the period from March to December 1994, 450 broiler carcasses and 400 broiler parts were investigated for *Salmonella*. The serological typing of the isolated *Salmonella* strains was carried out using specific, commercially available antisera (Behring, FRG). As a next step, 180 *Salmonella* isolates were investigated for their plasmid paterns.

Campylobacter isolates

From November 1994 up to January 1995, 380 broiler carcasses were investigated for the presence of *Campylobacter*. Serological typing of the *Campylobacter* strains which were detected in the investigation was carried out with API Campy System (bio Merieux, France). After differentiation, 40 *Campylobacter* isolates were investigated for their plasmid profiles.

Isolation of plasmids

The plasmid DNA was isolated from 2 ml of LB culture medium, which was further cultivated at 37°C according to the method of Kado and Liu (1981).

Electrophoresis of the plasmid DNA was carried out with 0.8%, horizontal agarose gels in TBE buffer and then stained with ethidium bromide.

Results

Results of the *Salmonella* isolates investigated in this study are summarized in Table 1. With a prevalence of 33% of all investigated strains, *S. enteritidis* was the most frequently isolated serovar. In second place was *S. virchow* with 28.4%, followed by *S. hadar* with 23.3%. *Salmonella bredeney* is a serovar which is rarely isolated from poultry meat, but which was nevertheless isolated fairly frequently during the present investigation. The usually more frequently isolated serovar *S. saint paul* was found in only 3.3% of cases. *S. berta*, is a serovar more frequently isolated from feedstuffs and less frequently from poultry meat and poultry meat products.

All strains listed in Table 1 are characterized by one or more plasmids (see poster). Prominent in this aspect are *S. enteritidis*, which has a plasmid of

37 Md, and *S. saint paul*, which has a large plasmid of 22 Md and smaller plasmids with molecular weights of 3.1, 2.4 and 1 Md, repectively. *Salmonella bredeney* has one plasmid of 55 Md and two small plasmids of 2.0 and 5.8 Md.

The *Campylobacter* isolates were serologically determined to be *Campylobacter jejuni*, biotype I (80% of all isolates) and *Campylobacter* coli (20% of all isolates). C. jejuni I only has small plasmids (1.0 and 1.8 Md). *C. coli* has a plasmid of 70 Md and two small plasmids with molecular weights of 2.0 and 8.0 Md, respectively.

Discussion

In recent years, determined attempts have been made to improve microbiological *Salmonella* diagnostics. The present study should be a piece of brickwork on this way. In it, it can be confirmed that it is possible to identify different serovars and to differentiate within serovars to a far finer degree using plasmid profile analysis. By being able to follow back identical plasmid profiles of *Salmonella* serovars from different sources, it is possible also to make use of plasmid profile analysis for epidemiological studies.

In poultry economics, *S. enteritidis* constitutes a special problem. 97% of the 33% *S. enteritidis* strains we isolated were characterized by a plasmid of 37 Md. Thus this plasmid is serovar-specific to a very high degree, and with it, it is possible to differentiate *S. enteritidis*.

S. hadar and *S. virchow* were frequently isolated from poultry meat parts. They are particularly characterized by small plasmids, which can also be utilized for epidemiological studies. *Salmonella bredeney* is completely different, frequently being characterized (57%) by the absence of any plasmids.

The plasmid profiles of *C. coli* and *C. jejuni*, with large plasmids on the one hand and particularly small plasmids on the other, allow the differentiation of *Campylobacter*. In the same way as most *Salmonella* serovars, it is possible to differentiate *Campylobacter* using specific plasmid profiles. Thus *C. jejuni* has mainly small plasmids, while it is characteristic for *C. coli* to have only one large plasmid.

Conclusion (Table 1)

The frequency of individual *Salmonella* serovars isolated from poultry meat (March to December 1994)

Salmonella servovar	Number investigated	Frequency in % of total	% with plasmids	% without plasmid
S. enteritidis	60	33,3	97	3
S. virchow	51	28,4	98	2
S. hadar	42	23,3	76	24
S. bredeney	19	10,5	43	57
S. saint paul	6	3,3	100	-
S. berta	2	1,1	100	-

Dr. V. Atanassova und Prof. Dr. Dr. Ch. Ring, Tierärztliche Hochschule Hannover, Zentrum für Lebensmittelwissenschaften, Abt. für Lebensmittelhygiene und -mikrobiologie, Bischofsholer Damm 15, D-30173 Hannover, Germany