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Plasmid Characterization of *Salmonella typhimurium* Transmitted from Animals to Humans

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The transmission of pathogenic bacteria from animals to humans is widely studied because of its public health importance. In this study, we show the transmission of *Salmonella typhimurium* from cattle which had received no growth-promoting antibiotics to humans who had direct contact with the ill animals. On one cattle farm, the veterinarian attending the sick animals became ill, and two other individuals living on the farm later developed salmonellosis. The strains isolated from both humans and animals at one farm were identical as to antibiotic susceptibility and phage type, and they were specifically traced by the presence of a common 24-megadalton plasmid. Restriction enzyme digests of this plasmid from both human and animal strains were identical. At another farm, tetracycline-resistant *S. typhimurium* strains possessing a different profile (eight plasmids) were isolated from both animals and humans. The tetracycline-resistant clone was also isolated from animals at a third farm, but with animals and humans having no known contact with those of the other two farms.

Salmonella infection appears to be one of the most common examples of an enteric disease that is transmitted from animals to humans. The transmission occurs both through food products, such as meat, dairy products, and eggs, and by direct contact between animals and humans through the fecal-oral route (2, 7, 11–13, 18, 20).

Epidemic or endemic salmonellosis in animals causes tremendous problems for the food industry and creates a potential health hazard for humans. Salmonellosis in humans can produce symptoms ranging in severity from gastric distress to death (19). Prophylactic work based on epidemiological surveys to find the reservoirs are given high priority. Outbreak strains have traditionally been traced by several different methods, including serotyping, biotyping, phage typing, and antimicrobial susceptibility testing. These methods were recently compared with plasmid profile analysis in an investigation of well-documented *Salmonella typhimurium* outbreaks (10). Plasmid profile analysis was found to be at least as good as the traditional methods, and this approach has been used in the characterization of other species (3, 17).

The addition of broad-spectrum antibiotics to animal feed has raised the question of whether such subtherapeutic doses might select resistant *Salmonella* strains which then can be transmitted to humans (9, 11). Antimicrobial resistance genes are often located on transmissible plasmids in *Escherichia coli* and other normal flora; antibiotic pressures can promote the transfer of these plasmids to other *E. coli* strains and to enteric pathogens such as *Salmonella* spp. It is of great interest to study animal isolates for the presence of these R plasmids.

We present here a study of *S. typhimurium* strains isolated from diseased cattle in four separate herds in Norway and of the human cases of salmonellosis associated with these farms. It should be emphasized that the addition of antibiotics to animal feed is not permitted in Norway.

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MATERIALS AND METHODS

Strains. Thirteen bovine strains of *S. typhimurium* were first isolated from diseased cattle at farm 1. Then, shortly later, three humans working at the farm became ill with diarrhea. *S. typhimurium* was isolated from the feces; three isolates were from a veterinarian inspecting the cattle, and two isolates were from two other persons (one isolate each) working with the cattle on this farm. The *S. typhimurium* strains investigated at farm 2 were first isolated from 1 diseased calf (11 calves died) and later from three humans who developed diarrhea. These persons were living at farm 2. At farm 3, *S. typhimurium* was isolated from a diseased calf, and two *S. oranienburg* strains were isolated from the owner of the farm. One bovine *S. typhimurium* isolate was from farm 4.

Antimicrobial susceptibility testing. The Sensi-Disc system (BBL Microbiology Systems, Cockeysville, Md.) was used to test all strains for resistance to the following antibiotics: ampicillin, carbenicillin, cephalothin, chloramphenicol, colistin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfadiazine, sulfamethoxazole, trimethoprim, and tetracycline.

Plasmid profiling. The strains were cultivated in 1 ml of Luria-Bertani broth overnight at 37°C in a roller drum. Samples (0.5 ml) of the broth were transferred to 1.5-ml Eppendorf tubes, the bacteria were harvested and lysed, and the plasmids were isolated by a modified Birnboim procedure as described previously (4, 14).

The lysates were electrophoresed in a 0.7% agarose gel with Tris-borate-EDTA buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA [pH 7.6]) at 35 mA (110 V) for 2.5 h at room temperature. The gels were stained with ethidium bromide for 20 min and destained with distilled water for 30 min. Photographs of the DNA bands were taken under UV

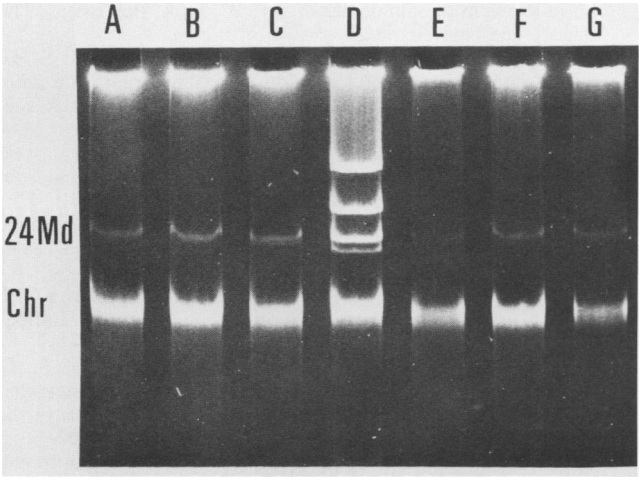

FIG. 1. Plasmid profiles of three human and three bovine *S. typhimurium* strains isolated at farm 1. Lanes: A through C, human isolates; D, control; E through G, bovine isolates. 24 Md, 24-MDa plasmid DNA; Chr, chromosomal DNA.

light exposure. *E. coli* K-12 strains containing the plasmids pDK9, RP4, and Sa were used as standards in each run.

Restriction endonuclease characterization. Plasmids from two human and two bovine isolates from farm 1 were analyzed by restriction endonuclease digestion. A phenol-chloroform extraction step was added before the alcohol precipitation step in the modified Birnboim procedure for plasmid samples that were characterized by a restriction enzyme. Restriction endonuclease digestion with *Hin*dIII (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) was carried out in accordance with the instructions of the manufacturer.

The samples were then electrophoresed in a 0.7% agarose gel with Tris-acetate buffer (40 mM Tris, 40 mM sodium acetate, 2 mM sodium EDTA [pH 8.2]) at 70 mA for 2 h. *Hin*dIII-cut fragments of phage lambda (Bethesda Research Laboratories) were used as standards and controls.

Phage typing. Phage typing of the *S. typhimurium* strains was performed with a collection of phages from the Centers for Diseases Control as described previously (1, 5).

Bacterial conjugation. The transfer of plasmids from two tetracycline-resistant *S. typhimurium* strains of bovine origin to *E. coli* K-12 strains was carried out at 37 and 25°C by the method described by Schlief and Wensinck (16).

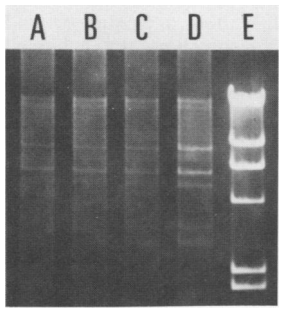

FIG. 2. *Hin*dIII endonuclease restriction profile of the 24-MDa plasmid. Lanes: A and B, human isolates from farm 1; C and D, bovine isolates from farm 1; E, phage lambda DNA.

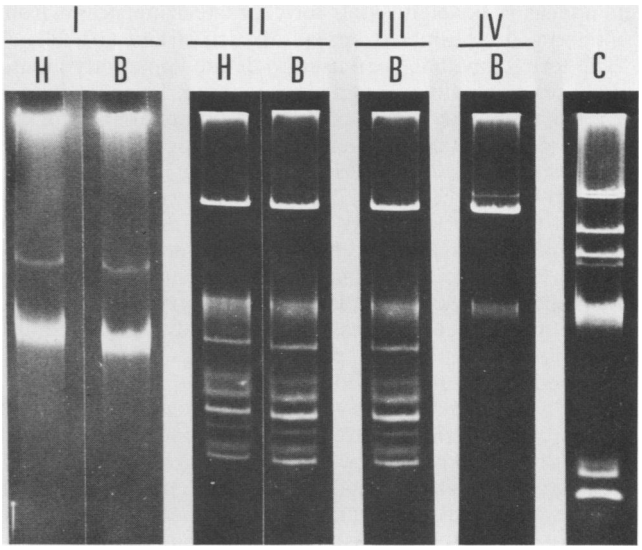

FIG. 3. Plasmid profiles of human (H) and bovine (B) *S. typhimurium* isolates from four different farms. Lanes: I, farm 1; II, farm 2; III, farm 3; IV, farm 4; C, control.

RESULTS

The five *S. typhimurium* strains isolated from the three humans at farm 1 had an identical plasmid pattern; one strain from each person is shown in Fig. 1. Each contained a single, low-intensity plasmid DNA band corresponding to a mass of 24 megadaltons (MDa). The low intensity may reflect a low copy number of the 24-MDa plasmid. The 13 bovine strains from the outbreak at farm 1, three of which are shown in Fig. 1, were all identical to the human strains. The restriction endonuclease digests of the 24-MDa plasmids are shown in Fig. 2. The identical digest fragments confirm common nucleic acid sequences and suggest that the human and bovine strains have a common origin. All strains from both animals and humans at farm 1 were susceptible to the antibiotics tested, and all belonged to phage type 14A.

The bovine strain isolated at farm 2 had a total of eight plasmids, the largest of which was 78 MDa (Fig. 3). This strain was resistant to tetracycline and belonged to phage type 8C. The three human strains from this farm were identical to the bovine strain and also identical to the strain isolated from a calf at farm 3 (Fig. 3). However, the human strains of *S. oranienburg* isolated from the owner of farm 3 did not have any plasmids and were susceptible to the antibiotics tested.

The single bovine strain isolated at farm 4 was susceptible to antibiotics, belonged to phage type 14A, and possessed only one plasmid of 62 MDa.

Attempts to conjugally transfer the tetracycline resistance genes from *S. typhimurium* strains isolated at farm 2 to *E. coli* K-12 strains were not successful.

DISCUSSION

Animal-to-human transmission of *Salmonella* strains is sometimes difficult to document with traditional epidemiological tools such as serotyping and biotyping. Many of the *Salmonella* serotypes are so commonly isolated (e.g., *S. typhimurium*) that epidemiologists need other means of subdividing the strains. Phage typing and plasmid profiling have provided the means for subgrouping *S. typhimurium*

and appear to be useful tools for characterizing strains from common sources and the spread of such strains (10, 20).

The term clone has been used to define apparently identical strains originating from a single source. Plasmid profiles have been found to be one of the best characteristics for the routine identification of bacteria originating from the same clone (15). For *S. typhimurium*, biotyping, serotyping, and antimicrobial susceptibility patterns did not provide sufficient information to clearly separate strains from different disease outbreaks. Phage typing and plasmid profiling were, however, found to be the most useful tools (10). Restriction endonuclease digests of plasmids can provide additional information. The strains tested in this study were generally susceptible to antibiotics, possibly because no antibiotics were used in the animal feed. Observations from the United States, where such additives are common, indicate that human *Salmonella* outbreaks originating from animal sources are often caused by resistant strains (11).

We have shown here that antibiotic-susceptible strains can be transmitted between animals and humans (farm 1) and also that tetracycline-resistant strains can do the same, although no antibiotics were added to the animal feed (farm 2). The time until the onset of disease was used to determinate the direction of the transmission (animal to human).

The tetracycline-resistant bovine strain similar to the human strains isolated at farm 2 was also isolated from a diseased calf at farm 3. Farms 2 and 3 are situated ca. 300 km apart, and there was no known contact between the farms. On farm 3, the owner with salmonellosis had *S. oranienburg* isolated both from blood and stools. This *Salmonella* species was at that time involved in a large outbreak possibly involving imported pepper in Norway (7, 13).

A multistate outbreak of *S. newport* in humans in the United States was traced to meat and further back to one herd by the presence of a single 24-MDa plasmid (9). This plasmid appears to be the same size as the plasmid found in the *S. typhimurium* isolates from farm 1. The restriction endonuclease profiles made with *Hin*dIII (9) were, however, different from those that we observed (Fig. 2). This shows the importance of doing restriction endonuclease digests when strains contain plasmids of similar molecular weights. The presence of a single plasmid cannot always be used to characterize a clone. Different serogroups of invasive *E. coli* possess a 140-MDa plasmid which is associated with the pathogenicity of these strains, but these strains are not from the same clone (8).

It may become important to know the function of different cryptic plasmids, especially regarding antibiotic resistance in *Salmonella* spp. Genetic probing with resistance genes should also provide very useful information in the study of transmission, not only of the bacterial clone, but also of resistance genes between enteric bacteria. Such studies have already been performed for the plasmids containing the toxin genes in enterotoxigenic *E. coli* (6).

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