Characterization of Salmonella enterica serovar Typhimurium isolates associated with septicaemia in swine

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Abstract

In this study we characterized, using genotyping and phenotyping methods, isolates (n=33) from septicaemia outbreaks in swine herds as well as isolates (n=33) recovered from healthy animals at slaughter. We determined the antimicrobial agents resistance profiles using 24 different antimicrobial agents by the disk diffusion on agar method, the phage type, the plasmid profiles and the PFGE profiles using XbaI and SpeI as restriction enzymes for each isolates. Resistance to as much as 10 antimicrobial agents was found in both categories of isolates. A greater number of PFGE genotypes was observed in isolates from septicaemia. Various phage types were identified in both groups of isolates. Among the DT104 phage type, many genetic clusters were identified. Analysis of plasmid profiles indicated that septicemic strains possess higher molecular weight plasmids than asymptomatic isolates. These results indicated that strains associated with septicaemia belong to various genetic lineages and suggest that virulence traits are associated with plasmid profiles of strains. Our results also suggest that the genetic diversity of Salmonella DT104 might be higher in North America if we consider results of similar studies in Europe.

Introduction

Infections caused by septicemic strains of Salmonella Typhimurium can be associated with significant mortality in mature pigs. Since previous studies showed persistence of strains in various tissues for many days following the infection, the presence of these strains represent as well a food safety concern. It is thus important to better characterize these isolates in order to understand pathogenesis of infection and develop appropriate control measures. The aim of this study was to characterize, using phenotypic and genotypic methods, isolates of S. enterica serovar Typhimurium associated with septicaemia in swine and to compare them to isolates recovered from clinically healthy pigs.

Material and Methods

Bacterial strains Salmonella isolates recovered from diarrheic and/or septicemic pigs and submitted for a necropsy were obtained from Dr. S. Messier (Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC), while those from clinically healthy pigs originated from previous studies in our laboratory (Letellier et al., 1999a; Rheault and Quessy, 2001). Isolates were serotyped at the MAPAQ (Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec) laboratory and phagetyped at the Health Canada Laboratory in Guelph (Ontario). The S. enterica serovar Typhimurium strain SL1344, previously described as highly invasive in in vitro invasion assays and fully virulent for mice, was provided by Dr. F. Daigle, Faculté de médecine, Université de Montréal, Montréal, QC. The Escherichia coli avirulent strain 8628B (provided by Dr. J. M. Fairbrother, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe, QC) was used as negative control. In addition to clinical signs, virulence of strains was also established according to their invasion rates on intestinal cells lines (Int-407).

Antimicrobial susceptibility testing. Susceptibility of the isolates to antimicrobial agents was determined by disk diffusion test on Muller-Hinton agar. Antibiotic tested were nalidixic acid 30 μg,
amikacin 30 µg, amoxicillin-clavulanic acid 30 µg, ampicillin 10 µg, cefoxitin 30 µg, cefotaxime 30 µg, cephalothin 30 µg, chloramphenicol 30 µg, ciprofloxacin 5 µg, gentamicin 10 µg, kanamycin 30 µg, streptomycin 25 µg, sulfamethoxazole 25 µg, tetracyclin 30 µg, trimethoprim-sulfamethoxazole 25 µg, apramycin 15 µg, bacitracin 10 IU, enrofloxacin 5 µg, erythromycin 15 µg, clindamycin 2 µg, neomycin 30 µg, quinupristin/dalfopristin 15 µg and vancomycin 30 µg. Results were interpreted according to the NCCLS guidelines for gram-negative enteric organisms.

Pulsed-field gel electrophoresis (PFGE). Chromosomal DNA plugs from an overnight bacterial culture on LB agar was digested with XbaI (recognition sequence TCTAGA) and ScaI (recognition sequence ACTAGT) (Invitrogen, Life Technology). PFGE performed on a horizontal agarose 1% gel for 13 h at 200 V, pulse time of 4-13.6 sec, at 14°C. Gels were stained with ethidium bromide and photographed on an UV transilluminator. The restriction endonuclease digest patterns were interpreted by considering migration distance and intensity of all visible bands.

Plasmids profiles. Bacterial culture were carried out at 37°C during 12 hours with agitation and low molecular weight plasmid profiles on 0.7% agarose gels were determined using QIAPrepR spin kit (Qiagen inc, Missisauga, Ontario, Canada) according to manufacturer’s guidelines.

Results

Serotyping and phagetyping. A total of 33 isolates of S. Typhimurium, the only serotype we have found so far in diseased animals, were recovered from septicemic pigs. When isolates were phagetyped, it was found that 33% (11/33) belonged to DT104 while the others belonged to various phage types. For the 33 isolates from clinically healthy pigs, the proportion belonging to DT104 was however similar.

Antimicrobials susceptibility testing. All isolates were resistant to at least 5 of the 22 tested antimicrobial agents. Resistance to as much as 10 antimicrobial agents were observed. Overall, there was no significant difference in antimicrobial resistance profiles in both group of isolates.

Pulsed-field gel electrophoresis (PFGE) A total of 15 different profiles were found with XbaI and 10 profiles with ScaI. Using XbaI, 11 different profiles were observed for strains from diseased animals compared to 7 for strains from healthy animals. Using ScaI, 9 profiles were identified in isolates from diseased animals while only 5 from isolates from healthy animals. Among the 11 S. Typhimurium DT 104 isolates from diseased animals, 6 different genotypes were observed.

Plasmid isolation and profiles. A higher number of low molecular weight (<10 000 bp) plasmids (average of 2.4 vs 1.3) was found in isolates from diseased animals. As much of 11 plasmids were observed on isolates from septicemic animals while a maximum of 3 bands were found in isolates from healthy pigs.

Discussion

In this study, different procedures were used in order to discriminate S. Typhimurium isolates recovered from septicemic animals to those from healthy pigs. Overall, as observed by other authors (Foley et al, 2006), a poor correlation was observed between the various typing methods. While it was not possible to find any differences between both groups of isolates regarding the antibiotic resistance profiles, we observed, with the convenient sampling scheme used in this study, a high genetic diversity in isolates from sick animals, suggesting that multiple genetic lineages are responsible for clinical outbreaks in swine herds. However, in a recent study (Perron et al, 2007) on the comparison, within the herds, of genetic variability of both groups of isolates, we observed a significantly higher genetic diversity in strains from asymptomatic animals, suggesting that once a virulent strains is established within a herd, this genetic lineage persist for a prolonged period. This high diversity was also observed in the S. Typhimurium DT 104 group of isolates, although it is generally accepted that isolates from this phage type are generally closely related (Baggesen et al, 2000).
When plasmid profiles of both groups of strains were compared, a higher number of low molecular weight plasmids was observed in isolates from septicemic pigs, suggesting that some virulence attributes may be linked to low molecular weight (< 10,000 bp) plasmids. It is well known that higher molecular weight plasmids are carrying some virulence factors of Salmonella (Bäumler et al., 2000). The elucidation of the exact role of these low molecular weight plasmids will need further works. We are currently characterizing these plasmids.

Conclusion

Salmonella Typhimurium isolates from septicemic pigs are genetically diversified and often multiresistant to antimicrobial agents. Results obtained in this study suggest that these isolates possess virulence attributes located on low molecular weight plasmids.

References


