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TITLE: Assessing the in vitro effect of wild-type lytic bacteriophages on Salmonella Minnesota field strains

Abstract Body: Salmonella Minnesota has been commonly detected in Brazilian broiler's farms. Today, the interest in the use of lytic bacteriophages is growing as an alternative to conventional feed additives to reduce colonization of broilers by non-typhoidal Salmonella at pre-harvest. This study aimed to determine the in vitro effect of three wild-type lytic phages (BRM 13312, BRM 13313 and BRM 13314) against S. Minnesota. In total, 32 S. Minnesota field strains isolated from drag swabs from commercial broiler houses in southern and midwestern Brazil were analyzed. First, these strains were characterized by antimicrobial susceptibility test against 11 antimicrobials using the disk diffusion method and XbaI-pulsed-field gel electrophoresis (PFGE) analysis. Next, the ability of phages to lyse S. Minnesota strains was individually analyzed using the spot lysis assay in triplicate. Bacterial strains were cultured at 37 °C for 18 h to obtain exponentially grown cultures. After dispensing 200 µL of each strain in 6 mL of nutrient broth-MgSO₄ containing 0.65% agarose, suspensions were mixed thoroughly and distributed onto nutrient agar containing 10 mM MgSO₄ to obtain overlay cultures. Ten µL of each phage was dispensed over the solidified agarose layer to give a multiplicity of infection of 1,000 phage particles to each bacterial cell. Salmonella Typhimurium ATCC 14028 and SM buffer were used as positive and negative controls, respectively. Plates were incubated at 37 °C for 18-24 h. Fisher exact test was used to compare the lytic effect of each phage on S. Minnesota strains. Overall, S. Minnesota strains showed a high diversity as revealed by 28 XbaI-PFGE patterns. One strain (3.1%) was fully susceptible to the tested antimicrobials. The remaining isolates showed resistance to amoxicillin with clavulanic acid (AMC); ceftiofur (CEF), streptomycin (STR), tetracycline (TET), and trimethoprim with sulfamethoxazole (TS), distributed in the following patterns: TET (34.4%), TET-TS (18.7%), TET-STR (15.6%), TET-CEF (9.4%), TET-STR-TS (9.4%), TET-CEF-STR (6.2%), and TET-AMC-CEF (3.1%). There was a difference in the effect of phages, as BRM 13314 produced clear lytic plaques in 28 (87.5%) S. Minnesota strains ($p \leq 0.05$). All tested strains were insensitive to the other phages. Phage-based products containing different bacteriophages intend to overcome bacterial resistance and lyse a wide variety of strains. Therefore, a cocktail replacing phages BRM 13312 and BRM 13313 would have a broader efficacy against Brazilian S. Minnesota field strains.

KEYWORDS: Salmonella, phage therapy, poultry, food safety.

CURRENT CATEGORY: Microbiology and Food Safety

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