

Staphylococcal gangrenous dermatitis in poultry: plasmid profile and its significance

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Twenty-six isolates of coagulase negative *Staphylococcus* sp. were isolated from two outbreaks of gangrenous dermatitis in poultry. Isolates exhibited multiple drug resistance. Frequency of resistance against gentamicin was highest (57.69%), followed by enrofloxacin (42.30%) and chloramphenicol (38.46%). All the isolates were sensitive to amoxicillin and ampicillin. The plasmid profile revealed the presence of three plasmids (15000, 1180 & 805 bp) in 9 (34.62%) and two plasmids (15000 & 545bp) in one strain only. Whereas, single plasmid (15600 bp) was recorded in 13 (50%) and none in 3 isolates (11.4%). In contrast to one plasmid (15600 bp) bearing strains all the eight isolates from second outbreaks harbour the single plasmid of molecular weight of 12763 bp. Plasmid profiling indicates involvement of different strains of coagulase negative *Staphylococcus* sp. in the outbreak and its significance in epidemiological studies.

Keywords: Gangrenous dermatitis; *Staphylococcus* sp.; antibiogram; plasmid profile

Introduction

Gangrenous dermatitis (GD) is one of the most important emerging diseases of poultry causing considerable economic losses to the industry. The disease is usually associated with the immunosuppressive diseases like infectious bursal disease, Marek's disease, aflatoxicosis and predisposing factors like poor ventilation, over crowding, poor hygiene etc., (Jordan, 1990). The disease is seen as a bacterial complication with clostridial and staphylococcal spp. (Shukla *et al.*, 1992) and *Escherichia coli*.

GD is characterized by areas of necrosis in the skin and underlying tissue, usually resulting in death. Any damage to the skin followed by a secondary bacterial infection can initiate the disease. Natural outbreaks of GD have been reported in 17 days to 20 weeks of age (Ficken *et al.*, 1988). The majority of reports of GD have been in broilers of four to eight weeks of age. The *Clostridia* sp. found in the soil and contaminated litter and the staphylococci found in the bird's skin and poultry house are the main causative agents. Many outbreaks of GD occur in the deep litter systems.

A plasmid is an autonomous self-replicating extra chromosomal DNA element. Plasmids are not essential for normal bacterial growth. Plasmids can carry antibiotic resistance or toxin genes (Ichiyama, *et al.*, 1991, Zhang *et al.*, 1998, Lange *et al.*, 2003). They can be transferred between bacteria of the same or different genera. Usually all functions required for plasmid transfer, including synthesis of pili, are encoded by genes on the plasmid. Thus, after transfer to a second host, these genes may enable a newly formed trans-conjugant to become a donor in another round of conjugation. This process may be repeated several times. Plasmids can also carry genes that code for functions other than transfer and replication such as toxin, adhesin, metabolic enzyme, and bacteriocin production.

Materials and methods

Fifteen and 11 isolates of coagulase negative *Staphylococcus* sp. isolated respectively from two outbreaks of gangrenous dermatitis in poultry were included in the study.

Antibiotic sensitivity test

In-vitro antibiotic susceptibility of individual isolates was determined by single disc diffusion method (Bauer *et al.*, 1966) with commercially available antibiotic discs (M/s. HiMedia Laboratories Ltd., Mumbai). The diameter of zones of inhibition was measured to nearest millimeter and interpretation was made as per the zone size interpretation chart provided by the manufacturer.

Plasmid profile of *Staphylococcus* sp.

Plasmid DNA isolation

The isolation of plasmid DNA was made as per the procedure of Sambrook *et al.* (2001). Briefly, overnight grown pure cultures was pelleted by centrifugation at 25000 g for 15 min. at -10°C . Pellet was treated with 200 μl of solution I (Glucose 50 mM, Tris 25 mM, EDTA 10 mM and lysozyme 4 mg/ml) and vortexed and kept on ice for 10 min. The cells were later treated with 200 μl solution II (NaOH 0.2 N, SDS 1%). The vial was inverted gently 4 to 5 times and kept at room temperature for 10 min. The ice-cold solution III 150 μl (5 M potassium acetate, glacial acetate acid, distilled water) was added and the vial was kept on ice for 15 min. The vial was centrifuged at 25000 g for 10 min. About 400 μl of supernatant was transferred to new vial.

A double volume of distilled ethanol was added to the supernatant, mixed thoroughly and stored on ice for 15 min and later centrifuged at 25000 g for 15 min. Pellet was resuspended in one ml of 70% ethanol and centrifuged at 25000 g for 5 min. The plasmid DNA pellet was air dried and dissolved in 50 μl of TE buffer. The plasmid DNA samples were stored at -20°C for further use.

Plasmid was separated by agarose gel electrophoresis (0.8% agarose containing 0.5 mg ethidium bromide in 0.5X Tris-EDTA electrophoresis buffer (TBE) at 1.5V/cm for 6 h and photographed under UV illumination.

Results and discussion

Coagulase negative *Staphylococcus* sp. isolated from two outbreaks of gangrenous dermatitis in poultry exhibited multiple drug resistance. The highest frequency of resistance was against gentamicin (57.69%), followed by enrofloxacin (42.30%) and chloramphenicol (38.46%). All the isolates were sensitive to amoxicillin and ampicillin. The plasmid profile revealed the presence of three plasmids (15000, 1180 & 805 bp) in nine (34.62%) and two plasmids (15000 & 545bp) in a strain only. Whereas, single plasmid (15600 bp) was recorded in 13 (50%) and none in 3 isolates (11.4%). Among one plasmid bearing strains, there was variation in the molecular weight of plasmid between the two outbreaks. Eight isolates of second outbreak harbored a single plasmid of 12763 bp in contrast to the five isolates from first outbreak. The antibiotic resistance pattern and plasmid profile pattern of the coagulase negative *Staphylococcus* sp. are shown in Fig. 1 and Fig. 2. respectively.

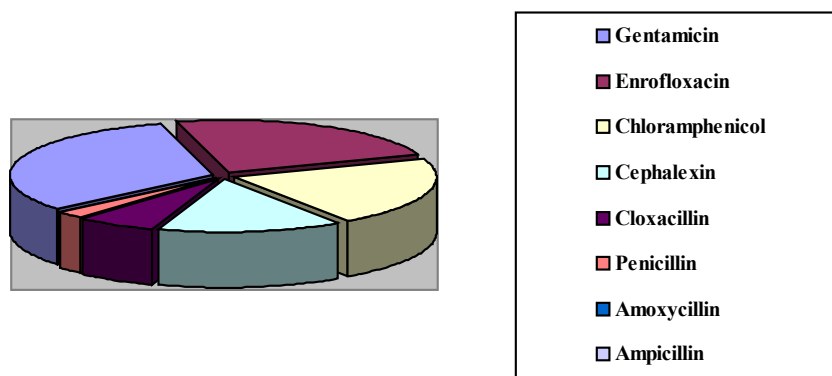


Figure 1 Antibiotic resistance pattern (per cent) of the *Staphylococcus* sp.

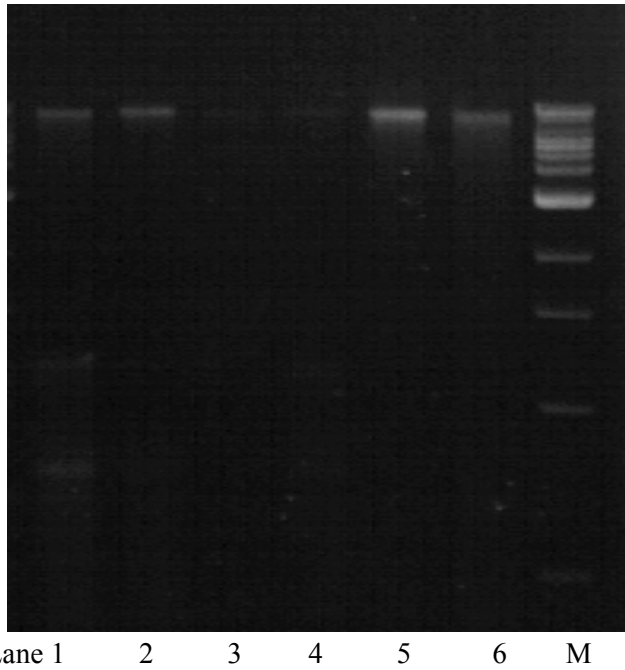


Figure 2 Plasmid profile of *Staphylococcus* sp. 3 plasmids (lane 10), 2 plasmids (lane 5), 1 plasmid (lane 2,3,4) & Molecular weight DNA marker MBD 20 (Banglore Genei, Banglore, India) (lane M).

In present study isolates from two outbreaks, differ in their antibiotic susceptibility and plasmid profile indicating involvement of different strains. The poor correlation between the plasmid profile and resistance against antibiotics indicate that possibly plasmids are responsible for other characteristics.

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