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Hemolytic Characterization and Antibiogram of Pathogenic Staphylococci Isolated from Quails

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Abstract

Pathogenic staphylococci generally cause dreadful foot infection in quails. A total of 220 samples were collected from ailing and dead quails and were examined for pathogenic characterization. Among the total samples collected 98 were post-mortem samples and rests were collected from ailing birds from bumble foot cases. Only 146 (i.e. 66.4%) positive strains of *Staphylococcus* spp. were detected in this study among which 84 (57.5%) were detected from post-mortem samples and rest 62 (42.5%) were from ante-mortem samples. Almost all the strains showed typical biochemical characteristics viz. indole and citrate negative, methyle red, Voges-Proskauer, catalase positive, but only 39 (26.7%) strains were detected to be pathogenic i.e. coagulase positive. Almost all (92.3%) the strains of pathogenic staphylococci showed hemolytic activity, mostly delta (δ) hemolysin (20.5%) and beta (β) hemolysin (17.9%) when tested on sheep and human blood agars. Antibiotic sensitivity testing of these pathogenic isolates revealed that almost all were sensitive to ciprofloxacing (94.8%), cephalexin (84.6%), gentamicin (79.5%), chloramphenicol and streptomycin (both 69.2%) but the antibiotic drugs like penicillin G (66.6%), nalidixic acid (56.4%), tetracycline (58.9%) failed to restrict their growth.

Key words : Hemolytic characterization, Antibiogram, Pathogenic staphylococci, Quails.

The poultry industry in India is developing speedily during last few decades in spite of various disease problems. The quail farming is now quite popular among the small farmers and in large organized farms due to their comparatively higher resistance to common infections, high acceptance of quail meat in the market, higher growth rate, quick return, easy maintenance. Though quails are more or less resistant from common diseases/infections as compared to other popultry birds there are few infections present in nature which can affect them also. Among these diseases of bacterial origin are quite common and can affect the productivity with ease. Among these Bumble foot is a dreadful disease of quails caused by *Staphylococcus* spp. (1). These bacteria cause foot infection, in co-ordination in gait, lameness in quails often leading to severe mortality. Therefore this condition can cause huge economic losses to the farmers. In view of these reasons the present study was undertaken to isolate the pathogenic staphylococci strains from ailing and dead quails, then to study their hemolytic activity in different blood agar media and test their drug sensitivity by the help of disc diffusion method (2) using 10 common antibiotics namely gentamicin, ciprofloxacin, cephalexin, chloramphenicol, streptomycin, penicillin G, nalidixic acid, tetracycline, amoxicillin and doxycycline to detect the drugs of choice against these pathogens.

Methods

In this present study for detection of pathogenic staphylococci, a total of 220 samples were collected from ailing and dead quails from different big organized and small private unorganized farms of south Bengal. The post-mortem samples (98) include visceral organs of quails died due to infections and the ante-mortem samples (122) collected from ailing birds (bumble foot cases) (3) include fecal samples, throat, nasal, cloacal swabs. All the samples after collection were put into enrichment broth and then transported to laboratory under ice cover for processing on same day or preserving at 4 C.

These samples were enriched in nutrient broth (Himedia) at 37 C for 14—18 hours for inoculating onto nutrient agar (Himedia) plates and then onto

Table 1. Hemolytic reactions showed by pathogenic staphylococci.

No. of strains	Hemolytic (36)														Non-hemolytic	
	α		β		δ		α-β		α-δ		β-δ		α-β-δ			
Coagulase +ve (39)	No. 3	% 7.7	No. 7	% 17.9	No. 8	% 20.5	No. 6	% 15.4	No. 6	% 15.4	No. 4	% 10.2	No. 2	% 5.1	No. 3	% 7.7

10% salt agar plates for obtaining tentatively pathogenic *Staphylococcus* spp. only. After proper incubation, obtained pure colonies of *Staphylococcus* spp. were morphologically examined by Gram's staining and biochemically characterized by different tests IMViC tests and catalase test (4). The tentative isolates were tested for pathogenicity by coagulase test with freshly collected rabbit plasma (1:10 with NSS) and young broth cultures of the isolated strains. The isolates showed positive result within 2—3 h were separated and considered for further testing only.

Test for hemolysin production by coagulase positive pathogenic staphylococci strains were carried out on 10% rabbit for alpha (α) hemolysin, sheep for beta (β) and human for delta (δ) blood agar plates. To determine the type of hemolysins, young broth cultures of the selected strains were streaked onto those sterile blood agar plates and then incubated at 37 C for 18—24 hours. The sheep blood agar plates were then refrigerated over night and again the results were recorded but for rest all plates the results were recorded at once. The zone of hemolysis were measued to detect the intensity of hemolytic reactions.

The antibiotic sensitivity of these pathogenic staphylococci strains were tested by dise diffusion method following Bauer et al. (2). Almost 5 ml of young broth cultures of the isolates were added to sterile nutrient agar plates and then the antibiotic discs were placed on them before incubation. The antibiotic used were penicillin-G, amoxicillin, ciprofloxacin, tetracycline, nalidixic acid, streptomycin, chloramphenicol, cephalexin, gentamicin and doxycycline. The results were interpreted with the help of electronic antibiotic zone reader.

Table 2. Results of antibiotic sensitivity tests. No. of isolates tested=39.

	Name of antimicrobial agents used	Sensitive strains No.	%	Intermediate strains No.	%	Resistant strains No.	%
1	Gentamicin	31	79.5	5	12.8	3	7.7
2	Doxycycline	12	30.8	5	12.8	22	56.4
3	Streptomycin	27	69.2	4	10.3	8	20.5
4	Tetracycline	10	25.7	6	15.4	23	58.9
5	Cephalexin	33	84.6	6	15.4	–	–
6	Amoxicillin	22	56.4	5	12.8	12	30.8
7	Nalidixic acid	4	10.3	13	33.3	22	56.4
8	Penicillin—G	2	5.2	11	28.2	26	66.6
9	Chloramphenicol	27	69.2	12	30.8	–	–
10	Ciprofloxacin	37	94.8	2	5.2	–	–

Results and Discussion

Out of 220 samples examined in this study, 146 (66.4%) isolates were detected to be positive for the presence of *Staphylococcus* spp. Among these isolates maximum were obtained from throat swabs (71.4%), cloacal swabs (87.5%), liver (76%) and spleen (92.4%). These findings matches with the findings of other workers like Das et al. (3) who isolated staphylococci from poultry sources. All these isolates showed typical results in biochemical tests viz. MR test, Voges-Proskauer test, nitrate reduction positive but negative to indole, citrate tests. These are positive to catalase tests also. These findings are in accordance with the findings of Laukova et al. (5) and Varshney et al. (6) who reported the staphylococci strains to be catalase, methyle red, VP positive and indole negative in their studies.

In this study only 39 (26.7%) of the positive isolates showed positive coagulase reaction and were pathogenic in nature. Others may be non-pathogenic. This result corelates with Menes et al. (7) and Bhalerao et al. (8) who reported almost 21—30% pathogenic strains earlier.

Almost all the strains (92.3%) showed hemolytic characters in this study. The frequency of δ hemolysin was the highest (20.5%) followed by β (17.9%) and α (7.7%) (Table 1). There strains showed combined hemolytic activity also such as α - β (15.4%), α

–δ (15.4%) and so on. Only three strains (7.7%) showed no hemolytic activity. These findings also corelate with that of Chatterjee and Nag (9), Bogni et al. (10) and Bedidi et al. (11).

In antibiogram it was revealed that the drugs like gentamicin (79.5%), cephalexin (84.6%), ciprofloxacin (94.8%), chloramphenicol could be used effectively against these pathogens but not penicillin G (66.6%), nalidixic acid (56.4%), tetracycline (58.9%) and doxycycline (56.4%) which failed to show sensitivity *in vitro* (Table 2). These results are similar with earlier workers (3, 12).

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