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BIOVARS AND ENTEROTOXIGENICITY OF STAPHYLOCOCCUS AUREUS ISOLATED FROM DISEASED BROILER - CHICKENS

BY

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INTRODUCTION

Staphylococcus aureus is widely distributed in nature in many species of warm - blood animals. There is an increasing evidence that strains carried by particular natural host have evolved sufficently so that specific ectotypes or biotypes can be considered as characteristic of particular host - adapted strains (Kloos, 1980). In this respect, most staphylococcal strains from humans are biovar A, those from poultry and swine are biovar B, and strains from rabbits are biovar D (Hajek and Marsalek, 1971). Other biovars E and F (from dogs, mink, horses, pigeons and foxes) are considered to be a separate species, Staphylococcus intermedius (Hajek, 1976 ; Meyer and schleifer, 1978). Despite the phenotype differences that from the basis of this biotyping scheme, all of these known biovars possess the well - known diagnostic characteristics of typical S. aureus such as coagulase positivity (rabbit plasma), production of heat stable DNase (thermonuclease) and possession of very high degree of DNA homology (Meyer and Schleifer , 1978).

S. aureus occurs in upper respiratory tract of normal , live poultry (Devriese et al., 1975). Chickens are commonly colonized by this organism during the first few days of life and its level increases to maximum at about the 7th week of life which is close to the usual slaughter - age for broiler (Thompson et al., 1980). On the other hand S. aureus was implicated in various poultry diseases such as septicaemia,

arthritis and pyogenic cutaneous infection and some strains were reported to produce enterotoxins (Gibbs et al., 1978 ; Raska et al., 1981 ; Gad El - Said et al., 1987).

The present paper deals with biovars and enterotoxigenicity of coagulase positive Staphylococcus aureus isolated from infected chickens.

MATERIAL AND METHODS

Samples:

A total of 226 swab samples was collected from septicaemic recently dead (65 cases) and diseased chicken - broilers with signs of suppurative arthritis (71 cases), suppurative skin lesions (50 cases) and respiratory troubles (40 cases), from various poultry farms in Egypt. The swabs were taken aseptically from heart blood, synovial fluid, pyogenic exudates and nasal discharge .

Isolation of coagulase positive S. aureus :

The fresh swabs were streaked on Baird - Parker medium that contained 75 mg/L polymyxin B (Sigma Chemicals Co., Ltd, Poole, England) recommended by Finegold and Sweeney (1961), incubated aerobically at 37°C for 24 - 48 hours for isolation and cultivation of coagulase positive S. aureus. Typical S.aureus colonies were picked up, and stored on BHI agar slants for further identification.

Identification and biotyping:

The isolated strains were checked for production of coagulase by using staphylase test (Oxoid Staphylase kit , Oxoid Ltd , England). The coagulase positive Staph. aureus strains were biotyped according to the scheme of modified Baird - Parker's classification proposed by Hajek and Marsalek (1971 & 1976). Characters examined were coagulase activity in human and bovine plasma (Cruickshank et al., 1975) α and B-haemolysins (Elek and Levy, 1950), crystal violet reaction (Meyer, 1967), staphylokinase production, using precipitated human plasma (Christie and Wilson, 1941) and tellurite reduction (Baird - Parker, 1962).

Production and detection of enterotoxin types :

The enterotoxins were produced by the identified isolates in sac culture method (Donnelly et al., 1967). They were detected in the culture supernatant using optimal sensitivity plate (OSP) method described by Robbins et al. (1974).

RESULTS

As shown in **(Table 1)**, coagulase positive staphylococci found in a total of 89 (39.38%) of 226 different cases of infected chicken - broilers. The highest percentage of coagulase positive staphylococci was recovered from suppurative skin lesions (52%) followed by cases of arthritis (42.26%), air - saculitis and bronchitis (37.5 %) and septicaemia (27.69%). The frequency of occurrence of enterotoxigenic isolates among coagulase positive staphylococci isolated from these cases was 25/89 (28.08%). Enterotoxigenic staphylococci recoverd from septicaemia, arthritis and air-saculitis and bronchitis of infected chicken - broiler ranged from 30 - 33.33% while in cases of suppurative skin lesion, enterotoxigenic staphylococci were found in only 5 out of 26 (19.23%) coagulase positive staphylococci. Of the 25 enterotoxigenic staphylococci isolates, 17 produced enterotoxin D, 3 produced enterotoxin A, 3 produced enterotoxins A + D and 2 were enterotoxin C producers.

Three biovars A , B and E were found in the coagulase positive staphylococci recovered from different cases of infected chicken - broilers **(Table 2)**. Of 18 strains of staphylococcal isolates from septicaemic cases, 6 belonged to biovar A and 12 to biovar B. All 30 staphylococcal isolated from cases of arthritis belonged exclusively to biovar B. Among the 26 staphylococcal strains recovered from cases of suppurative skin lesion, 14 belonged to biovar A,10 to biovar B and 2 to biovar E. Biovar B (11 isolates) and biovar E (4 isolates) could be identified among 15 staphylococcal isolates recovered from cases of respiratory troubles of infected chicken - broilers.

The determination of the biovars of enterotoxigenic staphylococcal isolates recovered from different cases of infected chicken broilers is shown in **(Table 3)**. Of 25 toxigenic

Table (1) : Incidence of enterotoxigenic isolates among coagulase positive staphylococci recovered from various cases of dead and infected broiler-chickens.

Cases	No. of samples tested	Coagulase positive isolates		Enterotoxigenic isolates		Enterotoxin type			
		No.	%	No.	%	A	C	D	A+D
Septicaemia	65	18	27.69	6	33.33	1	-	4	1
Suppurative arthritis	71	30	42.26	9	30.00	-	-	7	2
Suppurative skin lesion	50	26	52.00	5	19.23	2	1	2	-
Air-saculitis and bronchitis	40	15	37.50	5	33.33	-	1	4	-
Total	226	89	39.38	25	28.08	3	2	17	3

isolates from different cases, 3 produced enterotoxin A, 17 produced enterotoxin D and 3 produced enterotoxins A + D, and 2 produced enterotoxin C. The biovar A isolates from septicaemic cases (one isolate) and suppurative skin lesions (2 isolates) produced enterotoxin A. The biotype B isolates produced enterotoxin D (4 isolates with septicaemic cases), (7 isolates with arthritis) and (4 isolates with respiratory affaction) and enterotoxin A + D (one isoaltes with septicaemia) and (2 isolates with arthritis). The biovar E produced only enterotxin C (one isolate from suppurative skin lesion and one isolate from air - saculitis and bronchitis).

DISCUSSION

The results reported here demonstrate that coagulase positive staphylococci were recovered with an incidence of 39.38% from different cases of dead and infected broiler chickens with septicaemia, suppurative arthritis , suppurative skin lesions and respiratory affactions. These results substantiate the previous findings regarding staphylococcal infections recorded in chickens , Kuramasu et al. (1967), Harry (1967), Takeuchi and Suto (1973) and Gad El-Said et al. (1987).

Of 89 coagulase positive isolates recovered from different cases of dead and diseased broiler chickens, 25 (28.09%) proved to be enterotoxigenic ; a findings similar to that reported by Harvey et al. (1982), and lower than that recorded by Raska et al. (1981), 51.7% . In contrast, Shiozawa et al. (1980) found that only 3.8% of S. aureus isolates from healthy chickens were enterotoxigenic. The majority of enterotoxigenic strains 17/25 examined in this study produced enterotoxin D alone. Wieneke (1974) found nine of 13 enterotoxigenic strains isolated from raw chicken carcases and Shiozawa et al. (1980) reported six of 10 enterotoxigenic strains from poultry in Japan produced enterotoxin D alone. Our findings and that aforementioned correspond to that reported by Harvey et al. (1982) that the production of enterotoxin D may be characteristic of enterotoxigenic staphylococci of poultry origin. In contrast ,Hajek (1978) has reported that the production of enterotoxin D predominates among the ovine biovars .

Table (2): Biovars of coagulase positive S.aureus strains from different sites in broiler-chickens.

Cases	Coagulase		Haemolysin			Crystal violet reaction	Staphylokinase	Tellurite reduction	Biovars*		
	Hp	Bp	∝	B	Y	W	P			A	B	E
Septicaemia (18)	18	--	8	10	12	-	6	6	18	6	12	-
Arthritis (30)	30	--	5	25	30	-	-	3	30	-	30	-
Suppurative skin lesion (26)	24	2	11	15	10	2	14	14	24	14	10	2
Respiratory troubles (15)	11	4	2	13	11	4	-	2	11	-	11	4

() = No. of strains.

* = According to the Scheme of Baird7s classification modified by Hajek and Marsalek (1971, 1976).

A = Human origin. B = Poultry origin. E = Canine origin. (intermediate form)

Hp = Human. Y = Yellow pigment, W = White pigment,

Bp = Bovine plasma P = Purple pigment.

The present study has demonstrated that the coagulase positive staphylococcal isolates of biovar B (considered to be of poultry origin by Hajek and marsalek, 1971 , Gibbs et al., 1978) were implicated in cases of arthritis and biovar A (considered as human origin) and B were implicated in cases of septicaemia. Biovars A , B and E (considered to be canine origin) were found among the isolates recovered from cases of suppurative skin lesion and biovars B and E were implicated in cases of respiratory infections. These findings indicate that Staph. aureus biovar B (poultry Stains) seem to be the main cause of suppurative arthritis in infected chickens , while other biovars including B were most probably responsible for pathogenesis of other cases investigated in our study. Similar findings have been reported by Raska et al. (1981) who characterized staphylococci recovered from infected poultry (septicaemia , arthritis and pyogenic cuatenous infections) as well as water and food. On the other hand , Gibbs et al. (1978) reported that staphylococcal chicken - strains include biovar A (human) and other resembling biovar B that may however belong to a separate var. gallinarum (Witte et al., 1977). Moreover , S. aureus biovars E and F, include var. caninus which were found also in pigeons in addition to dogs (Hajek and Marsalek, 1969). This substentiates our findigns for Staph. aureus biovar E recovered from cases of suppurative skin lesions and respiratory affactions of infected broiler chickens. Regarding the relation between the enterotoxigenicity and biovars of the tested strains, biovar A produced enterotoxin A (3 strains), biovar B produced enterotoxin D (17 strains) and enterotoxins A + D) (3 strains) and biovar E produced enterotoxin C (2 strains). Few accounts of enterotoxin production and biotyping of poultry staphylococci have been met with in the available literature. Raska et al. (1981) reported that 34.5% of staphylococci isolated from infected poultry failed to produce enterotoxin, 49.4% produced enterotoxin D, 12.3% produced enterotoxin A and 3.8% produced enterotoxins A + D. Harvey et al. (1982) found that poultry strains (considered as biovar B) produced only enterotoxin D whereas human strains (considered as biovar A) produced enterotoxin A , C and D. Shiozawa et al. (9180) found that 2% of S. aureus isolates from apparently healthy chickens produced enterotoxin C. Moreover , Kato et al. (1978) reported that biovar E isolates (canine origin) produced enterotoxin C exclusively. However, Hajek and Marsalek (1973) found that animal strains of S. aureus (from hens ,

Table (3): Occurrence of S.aureus enterotoxins and biovars in relation to site of infection.

Cases	No.of isolates	No.of enterotoxgenic isolates	Biovars	Enterotoxins			
				A	C	D	A+D
Septicaemia	18	6	A	1	-	-	-
			B	-	-	4	1
Suppurative arthritis	30	9	B	-	-	7	2
Suppurative skin lesion	26	5	A	2	-	-	-
			B	-	-	2	-
			E	-	1	-	-
Air-saculitis and bronchitis	15	5	B	-	-	4	-
			E	-	1	-	-
Total	89	25		3	2	17	3

swine , cows , sheep , dogs , horses , mink and pigeons) were very infrequent producers of enterotoxin compared with human strains. Our results recorded here , and that of Raska et al . (1981) and Harvey et al. (1982) show that the production of enterotoxin D is rather common among staphylococci infected and or contaminate poultry.

From the hygienic stand point , attention should be paid towards the epidemiological aspects of S. aureus in poultry and type of enterotoxin produced by such strains , since Staph. aureus produced enterotoxins A and D are well known to implicated in human food poisoning (Bergdoll , 1970 ; Wieneke , 1974).

SUMMARY

The incidence of coagulase positive staphylococci in 226 samples collected from different cases of dead and diseased broiler chickens with septicaemia , suppurative artheritis , suppurative skin lesions and respiratory troubles, was determined and the biovars and enterotoxigenicity of the isoaltes were also investiagted. Coagulase positive S. aureus were found in 89/226 (39.38%) of the examined cases. Of 89 coagulase positive strains , 25 (28.08%) were enterotoxigenic , 17 isolates produced enterotoxin D , 3 produced enterotoxin A , 3 produced enterotoxins A + D , and 2 were enterotoxin C producers. Three biovars ; A (20 isolates , human origin) , B (63 isolates, poultry origin) and E (6 isoaltes , canine origin) were found in the tested coagulase positive staphylococcal strains. Of 20 biovar A isolates , 3 produced only enterotoxin A , of 63 biovar B isolates , 17 produced enterotoxin D and 3 produced enterotoxins A + D , and of 6 biovar E isolates , 2 produced enterotoxin C .

SUMMARY

1 . Baird - Parker , A.C. (1962): An improved diagnostic and selective medium for isolating coagulase positive staphylococci. J. Appl. Bacteriol. 25 , 12 - 19 .

2 . Bergdoll, M.S. (1970): Enterotoxins. In: Montie, T.C., Kadis, S., and Ajl, S.J.: Microbial toxins, Vol. III, pp. 265 - 326 , Academic Press, New York and London.

3 . Christie, R. and Wilson, H. (1941): A test of staphylococcal fibrinolysis. Aust. J. Exp. Biol. Med. Sci. **49**, 329-332.

4 . Cruickshank, R. ; Duguid , J.P. ; Marmion , B.P. and Swain, R.H.A. (1975): Medical Microbiology. The Practic of Medical Microbiology, 12th Ed., Vol. II Churchill Livingstone, Edinbrugh, London and New York.

5 . Devriese, L.A. ; Devos, A.H. and Damme, L.R. (1975): Quantitative aspects of the staphylococcus aureus flora of poultry. Poultry Science **54** , 95 - 101 .

6 . Donnelly, C.B. ; Leslie , J.E. ; Black, L.A. and Lewis , K.H. (1967): Serological identification of enterotoxigenic staphylococci from cheese. Appl. Microbiol. **15** , 1382 - 1387.

7 . Elek, S.D. and Levy, E. (1950): Distribution of haemolysis in pathogenic and non - pathogenic staphylococci. J. Path. Bact. **62** , 541 - 554.

8 . Finegold, S.M. and Sweeney, E.E. (1961): New seletive and differential medium for coagulase - positive staphylococci allowing rapid growth and strain differentiation. J. Bacteriol. **81** , 636 - 639 .

9 . Gad El-Said, W.A. ; Ismail, M. ; Ali, L. and Ata, N. (1987): Studies on staphylococcosis in chickens. J. Egypt. Vet . Med. Ass. **47** , (1&2) , 537 - 544 .

10. Gibbs, P.A. ; Patterson, J.T. and Harvey, J. (1978) : Biochemical charateristics and enterotoxigenicity of staphylococcus aureus strains isolated from poultry. J. Appl. Bacteriol . **44** , 57 - 74.

11. Hajek, V. (1976): Staphylococcus intermedius, a new species isolated from animals. Inst. J. Syst. Bacteriol, **26** , 401 - 408 .

12. Hajek, V. (1978): Identification of enterotoxigenic staphylococci from sheep and sheep cheese. Appl. Environ. Microbiol. **35** , 264 - 268 .

13. Hajek, V. and Marsalek, E. (1969): A study of staphylococci of bovine origin. Staphylococcus aureus var bovis. Zbl . Bakt. Hyg., I . Abt. Orig. **209** , 154 - 160 .

14. Hajek, V. and Marsalek, E. (1971): The differentiation of pathogenic staphylococci and a suggestion for their taxonomic classification. Zbl. Bakt. Hyg., I. Abt. Orig., A **217** , 176 - 182 .

15. Hajek, V. and Marsalek, E. (1973): The occurrence of enterotoxigenic staphylococcus aureus strains in hosts of different animal species. Zbl. Bakt. Hyg., I. abt. Orig, A **223** , 63 - 68 .

16. Hajek, V. and Marsalek, E. (1976): Evaluation of classificatory criteria for staphylococci. In: Jeljaszewicz, J. (ed): Staphylococci and staphylococcal diseases. G . Fischer Verlag, Stuttgart.

17. Harry, E.G. (1967): Some charateristics of staphylococcus aureus isolated from the skin and upper respiratory tract of domesticated and wild (feral) birds. Res. Vet. Soc., 8 , 479 - 489 .

18. Harry, J. ; Patterson, J.T. and Gibbs, P.A. (1982): Enterotoxigenicity of staphylococcus aureus isolated from poultry: raw poultry carcases as a potential food poisoning hazard. J. Appl. Bacteriol. **52** , 251 - 258 .

19. Kato, E. ; Kaji, Y. and Kaneko, K. (1978): Enterotoxigenic staphylococci of canine origin. Am. J. Vet. Res. **39**, 1771-1773.

20. Kloos, W.E. (1980): Natural populations of the genus staphylococcus. Ann. Rev. Microbiol. **34** , 559 - 592.

21. Kuramasu, S. ; Imamura, Y. ; Takizawa, T. ; Oguchi, F. and Tajima , Y. (1967): Studies on staphylococcosis in chickens. I. Outbreaks of staphylococcal infection on poultry farms and characteristic of staphylococcus aureus isolated from chickens. Zbl. Vet. Med. **14** B, 646 - 656 .

22. Meyer, W. (1967): Uber die Brauchbarkeit des Kristalviolett-Testes Zur Differenzierung Von Staphylococcus aureus - Stammen. Z. Med. Microbiol. Immunol. **153** , 158 - 168 .

23. Meyer, S.A. and Schleifer, K.H. (1978): Deoxyribonucleic acid reassociation in the classification of coagulase - positive staphylococci. Arch. Microbiol. **117**, 183-188.

24. Raska, K. ; Matejovska, D. ; Bergdoll, M.S. and Petrus, P. (1981): To the origin of contamination of foodstuffs by enterotoxigenic staphylococci. In: Staphylococci and staphylococcal infections. ed. Jeljaszewiez, J. pp. 381-385. G. Fischer Verlag, Stuttgart.

25. Robbins, R. ; Gould, S. and Bergdoll, M.S. (1974): Detecting the enterotoxigenicity of staphylococcus aureus strains. Appl. Microbiol. **28**, 946 - 950.

26. Shiozawa, K. ; Kato, E. and Shimizu, A. (1980): Enterotoxgeicity of staphylococcus aureus strains isolated from chickens. J.F. Prot. **43** , 683 - 685.

27. Takeuchi, S. and Suto, T. (1973): Biological characters of staphylococcus aureus isolated from diseased chickens. Nat. Inst. Anim. Hlth. Quart. **13** (3), 124 - 130.

28. Thompson, J.K. ; Gibbs, P.A. and Patterson, J.T. (1980): Staphylococcus aureus in commercial laying flocks : incidence and characteristics of strains isolated from chicks , pullets and hens in an integrated commercial enterprise. British Poultry Science **21** , 315 - 330.

29. Wieneke, A.A. (1974): Enterotoxin production by strains of staphylococcus aureus isolated from foods and human beings. J. Hyg. Camb. **73** , 255 - 262 .

30. Witte, W. ; Hummel, R. ; Meyer, W. ; Enner, H. and Wcndrak, R. (1977): Ecology of staphylococcus aureus : Characterization of strains from chicken. Zeitschrift Allg. Microbiol. **17** , 639 - 646 .