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ISOLATION OF COMMON CONTAMINANTS FROM VETERINARY BIOLOGICAL PRODUCTS AND THEIR SIGNIFICANCE

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Abstract

Eight-hundred and thirty three (833) samples of biological products ("315" live attenuated egg adapted vaccines, "193" live attenuated tissue culture adapted vaccines, "268" inactivated vaccines, "12" antigens, "9" anti-tetanic serum, 11 normal serum (neoborn calf serum, fetal calf serum, horse and swine serum and "25" physiological saline) were screened against common contaminants (Bacterial, Fungal and Mycoplasmal), and antibiotic sensitivity to these contaminants were determined. The results revealed that, out of the total number of samples (833), 18 samples (2.2%) were contaminated with various types of contaminants (anthracoid "6", proteus "5", fungus "2", *staphylococci* 2, *E.coli* 1, *Mycoplasma* 1 and *streptococci* 1). Concerning the results of antibiotic sensitivity test, it was clear that anthracoid, proteus, fungi, *E.coli* and *Mycoplasma* were sensitive to chloramphenicol, P-nitrophenyl glycerol, mycostatine, amoxycillin, and lincospectin, respectively, with varying degrees. Staphylococci and streptococci contaminants were sensitive to benzyl penicillin. By application of this study, it is possible to control the contaminants which interfere the production of different veterinary biologics.

INTRODUCTION

Veterinary biological products such as sera and vaccines are of the most important means used for controlling epidemics in animal and poultry production projects. The masive production of such biological products is objected with some difficulties represented in their production, storage, and thus, giving great demands of the application of criteria to ensure good keeping quality of such biologicals.

Major changes are being made to the process of registration and regulations dealing with immunological veterinary products in USA and in European community. During the past decade, there had been no discussions on harmonizing regulatory requirements for veterinary biologicals in Egypt. But, over the last few years, much work has been undertaken to overcome this problem.

Although there has been no harmonization between the different countries in the regulations used for licensing veterinary biologics yet, all of them require sera and vaccines to be sterile, safe consistency. It was the aim of this study to isolate and identify the most common bacterial, mycoplasmal and fungal contaminants encountered during the sterility test of veterinary biologics with determination of their antibiotic sensitivity and their significance (Code of Federal Regulations, 1985).

MATERIALS AND METHODS

Material

I. Vaccines examined

"315" live attenuated egg adapted vaccines, "193" live attenuated tissue culture adapted vaccines and "268" inactivated vaccine were examined for sterility test against contamination with bacterial fungal and mycoplasmal contaminants according to Code of Federal Regulations (1985).

II. Antigens and sera examined

"12" antigen samples, "9" antitetanic, "11" sera of neoborn calf serum, fetal calf serum, horse, swine sera and "25" physiological saline solutions were examined for sterility test according to Code of Federal Regulations (1985).

III. Detection of bacterial and fungal contaminants (Code of federal regulations, 1985)

The examined products were inoculated in sterile nutrient agar, nutrient broth, blood agar and MacConkey agar media and incubated at 37°C for 24 hours for detection of contaminats with aerobic bacterial and enterobacteriacae.

The tested products were also inoculated in sterile media of thioglycollate broth, coocked meat broth and blood agar, then, incubated in anaerobic jar at 37°C for 72 hours for detection of anaerobic bacterial contaminants and their toxins.

The tested products were cultured on sabauroud's dextrose agar plates and sabauroud's dextrose broth, then, incubated at 25°C for 3 days for detection of fungal contaminants.

IV. Detection of mycoplasmal contaminants (Code of federal regulations, 1985)

The tested products were inoculated in Frey' medium supplemented with nicotinamide adenine dinucleotide (NAD), then, all plates were incubated in a high humidity, 5% CO2 at 37°C for 14 days, and examined with a stereoscopic microscope, according to Frey *et al.*. (1968). Also, growth inhibition test was done according to Cullen and Anderson (1974) and Erno and stipkovits (1973).

V. Identification of their contaminants (Carter and Cole, 1990)

The common contaminants (bacterial, fungal and mycoplasmal) were identified with colonial morphology, Gram's staining, biochemically by the API system testing.

Also, antibiotic sensitivity was done on these contaminants according to Kopp *et al.* (1966) and Senior (1978).

RESULTS AND DISCUSSION

When national licensing authorities receive an application which convinces them of the suitability of a vaccine, such vaccine must pass a series of quality control tests before a permission is given to the company to sell or supply the product. In general terms, the directives require vaccines to be safe, sterile, and efficient. Sterility tests are very important not only for making sure of the good processing techniques used in vaccine production, but also to have good evidence that this vaccine (especially living vaccines) will not introduce another pathogen to the vaccinated animals or birds.

In this study, 833 samples of biological products (315 live attenuated egg adapted vaccines, 193 live attenuated tissue culture adapted vaccines, 268 inactivated vaccines, 12 diagnostic antigens, 9 anti-tetanic serum, 11 serum amples and 25 physiological saline) were screened for the presence of contaminants (Table 1).

Microbiological examination of these samples revealed that, 18 samples (2.2%) (Tables 2, 3, 4, 5, 6 and 7) were contaminated with various types of contaminants which were identified biochemically as anthracoid (Clarridge, 1987), proteus (Brenner, 1984), fungus (Tilton and McGinnis, 1987), staphylococci (Barnes, 1987), *E.coli* (Finegold and Baron, 1986), streptococci (Sneath, 1986) and mycoplasma (Bradbury and Kleven 1987). These contaminated samples, as shown in Table

8 are recorded as anthracoid (6), proteus (5), fungus (2), staphylococci (2), *E.coli* (1), streptococci (1) and mycoplasma (1).

These contaminants were sensitive to chloramphenicol, P. nitrophenyl glycerol, mycostatin, benzyl penicillin, amoxycillin and lincospectin with various degrees according to the type of contaminants either bacterial, fungal or mycoplasmal (Table 9).

This finding was valuable, not only for ensuring the good quality of the biological product, but also for using some of these antibiotics during the manfacturing of some vaccines esspecially the adapted vaccine, tissue culture vaccines and living attenuated vaccine. This addition of these antibiotics prevents uncontrolled contamination during vaccine processing.

Table 1. Percent of sterile and contaminated samples examined by sterility tests in one year.

Samples	Total No. of each samples	No. of sterile samples	% of sterile samples	No. of contaminated samples	% of contaminated samples
Living attenuated vaccine adapted on egg	315	306	97	9	3
Living atenuated vaccine adapted on tissue culture	193	192	99.5	1	0.5
Inactivated vaccine	268	266	99	2	1
Antigens	12	12	100	-	0
Serum	11	11	100	-	0
Antitetanic serum	9	9	100	-	0
Physiological saline	25	19	76	6	24
Total	883	815	97.8	18	2.2

Table 2. Identification of staphylococci organism isolated from contaminated biological products and identified by using API system No. V3. 1.

Type of organism	D-Glucose	D-fructose	D-Maltose	Maltose	Lactose	Potassium nitrate	PAL	VP	Flurescent reaction	Raffinose	Xylose
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	-	-
Staphylococcus saprophyticus	+	+	-	+	+	-	-	-	-	-	-

PAL : B-naphthyl-acid phosphate (Alkaline phosphatase).

VP : Sodium pyruvate (Acetyl-methyl-carbinol production).

Table 3. Identification of staphylococci organism isolated from contaminated biological products and identified by using API system No. V5. 1.

Type of organism	Pyruvate (VP)	Hippurate (Hip)	Alkaline phos-phatase (PAL)	Arginine dihy-drolase (ADH)	Ribose	Lactose	Trehalose
Streptococcus pyogenes	-	-	+	+	-	+	+

Table 4. Identification of spore forming Gram possitive aerobic bacteria which isolated from contaminated biological products.

Type of organism	Colonial pig-ment	Motility	Glucose	Catalase	Maltose	Lactose	Xylose
Saprophitic anthracoid	White colong	+	+	+	+	-	-

Table 5. Identification of proteus and E.coli organisms isolated from different types of contaminated biological products and identified by API system No. 10E.

Type of organism	Glucose	Arabin-ose	Indol	Urease	Sodium citrate	ONPG	Typtophane desaminase
Proteus vulgaris	+	-	+	+	+	ND	+
Escherichia coli	+	+	+	-	-	+	ND

ND : Not Done.

PNPG : Ortho-nitrophenyl galactoside.

Table 6. Identification of the genus mycoplasma isolated from different types of contaminated biological products.

Type of organism	Glucose catabo-lism	Manose catabolism	Tetraz-olium	Digitonin disc sensi	NAD requi-rment	Growth inhibition test
Mycoplasma bovis	-	-	+	inhibition zone around the disc	Growth without NAD	Anti MB sera paper discs zone of inhibition

Table 7. Identification of proteus and *E.coli* organisms isolated from different types of contaminated biological products and identified by API system No. 10E.

Type of organism	Glucose	Arabin-ose	Indol	Urease	Sodium citrate	ONPG	Typtophane desaminase
Proteus vulgaris	+	-	+	+	+	ND	+
Escherichia coli	+	+	+	-	-	+	ND

ND : Not Done.
PNPG : Ortho-nitrophenyl galactoside.

Table 8. Identification of the genus mycoplasma isolated from different types of contaminated biological products.

Type of organism	Glucose catabo-lism	Manose catabolism	Tetraz-olium	Digitonin disc sensi	NAD requi-rment	Growth inhibition test
Mycoplasma bovis	-	-	+	inhibition zone around the disc	Growth without NAD	Anti MB sera paper discs zone of inhibition

Table 9. Results of Identification of fungi isolated from different types of tested biological and non biological products.

Total No. of tested samples	No. of contami-nated samples	Suspected species of fungal contaminant
833	2	* *Penicillium sp.* * *Fusarium sp.*

Table 10. Number and type of contaminants in each type of products examined.

Type of examined products	Type of bacterial contaminants						Fungi	Mycoplasma
	Aerobic bacteria							
	Anaerobic bacteria	Anthr-acoid	Proteus	Slaphylo cocci	E.coli	Strepto-cocci		
Living egg adapted vaccine	-	-	-	-	1	-	-	-
Living tissue culture adapted vaccine	-	-	-	-	-	-	-	-
Inactivated vaccines	-	-	-	-	-	-	-	-
Diagnostic antigens	-	2	1	-	-	-	-	-
Antitetanic serum	-	1	2	1	-	-	-	-
Normal serum	-	-	-	-	-	-	-	1
physiological saline	-	3	2	1	-	1	2	-

Table 11. Aritibiotic sensitivity discs against bacterial, fungal and mycoplasmal contaminants.

Type of antibiotic disc	Type of bacterial contaminants															Fungi			Mycoplasma		
	Anaerobic			Proteus			Staphylococia			E.coli			Streptococi								
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Chloramphenicol	6	-	-	-	1	-	-	2	-	-	1	-	-	1	-	-	-	2	-	-	1
Amoxycilin	-	-	-	-	5	-	-	2	-	1	-	-	-	1	-	-	-	2	-	-	1
Lincospectin	-	6	-	-	-	5	5	-	2	-	-	1	-	-	1	-	-	2	1	-	-
Mycostatine	-	6	-	-	-	5	5	-	2	-	-	1	-	-	1	-	-	-	-	-	1
P-nitro-phenyl glcerol	-	6	4	4	1	-	-	-	2	-	-	1	-	-	1	2	-	2	-	-	1
Benzyl penicillin	-	4	-	-	3	2	2	-	-	-	-	1	1	-	-	-	-	2	-	-	1

S : Sensitive. I : Internediate. R : Resistance.

REFERENCES

1. Barnes-Pallesen, F.D. 1987. Laboratory and Field Handbook on Bovine Mastitis. Ft. Atkinson, Wisconsin, W.D. Hoard and Sons Co., National Mastitis Council, P. 25.

2. Bradbury, J.M. and S.H. Keven. 1987. Avian mycoplasmas: detection and control update. Israel J. Med. Sci., 23 (6): 771-772.

3. Brener, D.J. 1984. The family Enterobacteriacae Rahn. 1937. In Krieg, N.R. (Ed.): Bergey's Manual of Systematic Bacteriology, Vol. 1. Baltimore, Williams and Wilkins.

4. Carter, G.R. and J.R.Jr. Cole. 1990. Diagnostic procedures in veterinary bacteriology and mycology. 5th Edition, Academic Press, Inc. Harcourt Brace Jovanovich, Publishers.

5. Clarridge, J.E. 1987. Gram-positive bacilli. In Howard, B.J. et al. (Eds.): Clinical and Pathogenic Microbiology. Washington, D.C. Mosby, Chapter 21.

6. Code of Federal Regulation. 1985. Animals and Animal Products, Parts 1 to 199. Revised as of January 1, 376-385. Published by the Office of the Federal Register as a special Edition of the federal Register.

7. Cullen, G.A. and M.F. Anderton. 1974. Comparative efficiency of some rapid agglutination antigens for M.gallisepticum infection. Avian Path., 3: 89-103.

8. Erno, H. and L. Stipkovits. 1973. Bovine mycoplasmas. Cultural and biochemical studies. Acta Vet. Scand., 14: 450-463.

9. Finegold, S.M. and E.J. Baron. 1986. Enterobacteriaecae. In Bailey and Scott's Diagnostic Microbiology, 7th ed. St. Louis, Missouri, Mosby.

10. Frey, M.L., R.P. Hanson. and D.P. Anderson. 1968. A medium for the isolation of avian mycoplams. Am. J. Vet. Res., 29: 2163-2171.

11. Kopp, R., J. Muller and R. Lemme. 1966. Inhibition of searming proteus by sodium tetradecyl sulphate, B-phenyl ethyl alcohol, and P-nitrophenyl glycerol. Applied Microbiology, 14: 873-878.

12. Senior, B.W. 1978. P-nitrophenylglycerol. A superior antiswarming agent for isolating and identifying pathogens from clinical material. J. Med. Microbiol., 11: 59-61.

13. Sneath, P.H.A. 1986. (Ed-in-Chief): Bergey's Manual of Systematic Bacteriology, Vol. 2. Baltimore, Williams and Wikins.

14. Tilton, R.C. and McGinnis, M.R. 1987. Dernatophytes. In Howard B.J. (ed.); Clinical and Pathogenic Microbiology. Washington, D.C., Mosby.

عزل الملوثات الشائعة في المنتجات الحيوية البيطرية ومعرفة طرق التغلب عليها

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المعمل المركزي للرقابة علي المستحضرات الحيوية البيطرية-مركز البحوث الزراعية - جيزة مصر.

تم في هذه الدراسة فحص ٨٣٣ عينه من المنتجات الحيوية البيطرية (٣١٥ لقاح مستضعف مؤقلم في بيض، ١٩٣ لقاح حي مستضعف مؤقلم علي خلايا، ٢٦٨ لقاح متبط، ١٢ أنتجين تشخيصي، ٩ مصل مضاد لمرض الكزاز، ١١ مصل طبيعي " عبارة عن مصل لعجول حديثي الولادة - مصل أجنحة عجول - مصل خيول ومصل خنازير، ٢٥٦ محلول ملح فسيولوجي) وذلك لإثبات خلوها من الملوثات الشائعة البكتيرية والفطرية والميكوبلازما واختبار حساسيتها للمضادات الحيوية المختلفة.

وأوضحت النتائج أن من أجمالي ٨٣٣ عينه ١٨ عينة "٢,٢ ٪ كانت ملوثه علي النحو التالي (٦ بميكروب شبيه العصريات، ٥ بميكروب البروتيو، ٢ بالفطريات، ٢ بالميكروب العنقودي المكور، ١ بميكروب القولون المعدي، ١ بالميكوبلازما و ١ بميكروب السبحي المكور).

وباختبار حساسية هذه الميكروبات وجد أن ميكروب شبيه العصويات، البروتيوس والفطريات، التهاب القولون المعدي والميكوبلازما كانت حساسيتهم للكلورامفينكول، بي نيتروفينيل جليسرول، ميكوستاتين، أموكسي سيللين ولينكوسبكتين علقي التوالي وذلك بدرجات متفاوته بينما الميكروبات العنقودية المكورة والسبحية المكورة كانت حساسيتها للبنزيل بنسللين.

وبتطبيق هذه الدراسة يمكن السيطرة علي الملوثات التي تعوق انتاج المنتجات الحيوية البيطرية المختلفة.