تقدير قوة التوكسينات التى تفرزها الكورينى أوفيس

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درست القدرة على افراز التوكسينات الخارجية لميكروب الكورينى أوفيس بواسطة ثلاث طرق مختلفة وقد أستخدم أختبار (أقل جرعة تتفاعل / مللى) فى جلد الأرنب وقد أظهرت الدراسة أن ثمانية عترات من العترات المحلية أعطت توكسينات خارجية بينما كانت هناك عترتين لا تعطى توكسينات خارجية .

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POTENCY OF TOXIN PRODUCTION AND DETERMINATION OF MINIMAL REACTING DOSE OF LOCAL STRAINS OF C. OVIS.

(With 2 Tables and One Figure)

By

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(Received at 12/12/1979)

SUMMARY

Ten C.ovis strains isolated from different localities in Egypt were tested for toxin production by three different methods. Measuring of the potency of the prepared toxin was applied by determination of minimal reacting dose (MRD/ml) in rabbit skin sensitivity test.

The results indicated the recovery of 8 toxinogenic strains from the local strains examined while 2 were non-toxinogenic. The modified method produced higher level of C.ovis toxin which varied from 20-640 minimal reacting dose/ml.

The modified method for preparation of the toxin was carried out by inoculating the examined strains on veal infusion agar and icubated for 24 hours at 37°C. Then the resultant growth was transferred to the modified digest medium and incubated for 7 days.

INTRODUCTION

On bacteriological study of C.ovis strains, it was found that certain strains produce a haemoglobinurea or icterus in experimentally infected sheep due to its biproducts but failed occasionally to produce acute intoxication (DANIS and AUSTIN,1932; CARNE,1939 and 1940).Moreover MADDY (1953) and FRASER (1961) recorded that C.ovis leucocidin and haemolysin, were active against g.pig, rabbit, horse and sheep red blood

corpusle. It was thermostable and linked to bacterial cell. Subsequently DOTY et al. (1964), FRASER (1964) and JOLLY (1966) stated that a curious or potent exotoxins were present in C.ovis culture and/or supernatant which on injection might be lethal to many animal species. Local area of inflammation and neccosis, may be resulted but it did not cause acute intoxication with death in case of natural infection by the organism i.e. C.ovis. Therefor DOTY et al. (1964) recommended the use of the skin reaction for demonstration of the C.ovis toxin or for detection of C.ovis infection by demonstration of the antitoxin in the serum by means of rabbit-skin sensitivity test.

LOVEL and ZAKI (1966) studied the growth products of C.ovis and found that 97.33% of C.ovis strains recovered from different animals species produced lethal toxin which killed white mice within 4 days and inhibited the effect of staphyloccal B-lysin. They concluded that these activities appeared due to one or two or more diffusable substance which were strongly related as all were inhibited by an appropriate antiserum. At the sametime, SMITH (1966) clamied that in suitable media a soluble heatlabile toxin closely bound to the bacterial cell of C.ovis was slowly released and when injected intradermally in g.pigs or rabbit produce a zone of flushing with central necrotic area.

The aim of this investigation is to study the toxin production by 10 local strains of C.ovis and to recognize the most siutable method for high toxin production.

MATERIAL AND METHODS

Ten C.ovis strains were obtined from the collection of Animal Research Institute, Dokki, Cairo. They were isolated by Aerobic Bacteria Section from cases suffered from caseous lymphadenitis in sheep and goat (Strain C.o.1) from different localities in Egypt.

The toxin was prepared by the three following methods to compare the efficiency of each of them in towin production.

TOXIN PRODUCTION C. OVIS.

1) DOTY et al. METHOD (1964): C.ovis was grown on veal infusion agar at 37°C for 24 hrs. The resultant growth was transferred to nutrient broth distributed in Roux bottles, each contained 180 ml. The Roux bottles were agitated to ensure complete mixing and placed at incubator at 37°C for 7 days. The toxin was harvested after placing the flasks in the refrigerator overnight to allow the bacterial cell to settle.

2) LOBELL and ZAKI METHOD (1966): The examined strains were cultivated on blood agar for 48 hrs at 37°C. then 2-3 loopfulls were inoculated in 25 ml volumes of digest medium of carne (1940) in flat medicine bottles. The bottles were incubated at 37°C for 7 days in standing position.

3) Modified Method: The examined strains were cultivated on veal infusion agar for 24 hrs. at 37°C, then the growth was harvested and transferred in modified digest medium of LOVELL and ZAKI (1966) in Roux bottle contained 180 ml, then incubated for 7 days at 37°C.

The toxin was harvested after overnight refrigeration in DOTY et al. (1964) method or directly in the other two methods by centrifugation at 4000 r.p.m. in sterile tubes for 20-30 minutes. The supernatant was kept at 4°C with or without covering with a layer of totuol, while the sediment was cultured to check purity.

Titration of toxin by Rabbit-skin sensitivity test DOTY et al. 1964 :

The quantity of exotoxin produced by each of the aforementioned methods was determined by the application of rabbit skin sensitivity test. The test was applied by shaing the back of Baladi rabbit. The shaved back was marked of into 8 squares of approximately 9.0 cm^2 for each (Fig. 1). A series of two fold dilution of toxic supernatant or broth was made with equal amount of saline solution (0.2 ml of toxin + 0.2 ml of saline) and 0.2 ml of each dilution was injected intradermally

in each square as in Table (1).

Table 1: Schedule of intradermal injection in skin of rabbit with C.ovis toxins and its dilution to detrmine the minimal reacting dose / ml.

Square No.	Dilution of Toxin*	End Titre	MRD/ml
1	Crude toxin	Conc	
2	First dilution of toxin	1 : 2	
3	2 nd " " "	1 : 4	
4	3 rd " " "	1 : 8	
5	4 th " " "	1 : 16	
6	5 th " " "	1 : 32	
7	6 th " " "	1 : 64	
8	7 th " " "	1 : 128	

* Serial dilutions with equal amount of saline.

Results

Application of skin sensitivity test on 30 balady rabbit to determine the minimal reacting dose (MRD/ml) of toxins produced by using the different methods was investigated. The occurrance of a graded series of skin reactions ranges from a large swelling with marked necrosis termination to a red flush in a small area that disappeared completely with 24 to 48 hours was noticed. Such skin reaction was observed by 8 strains only, while the other two strains (C.o. 2 and 5) did not have the power to intiate any skin teaction and could be considered as non-toxinogenic strains.

Generally it was found that there were variations in the potency and the minimal reacting dose of the toxins prepared by the three methods. The toxins prepared by the DOTY *et al.* method showed the mildest skin reaction, where the MRD/ml of 6 strains was 20 and for two strains

was 40 (Table 2). The toxins prepared by LOVLL and ZAKI method gave skin resction in the form of red flush in a small area as the MRD/ml in 7 strains was 40 except one strain produced only slight reaction as its MRD/ml was 20. On the other hand, the modified method produced toxins of 20 MRD/ml by 4 strains, toxins of 40 MRD/ml by strain C.o.3, and toxins of 80, 160 and 640 by strains C.o. 10, Co. 8 and C.o. 9 respectively with the appearence of severe skin reaction (Table 2).

Table 2: The potency of C.ovis strains for toxin production by using different methods.

Strain No.	Toxin titres as produced by technique of			the amount of
	Doty et al. (1964)	Lovell & Zaki (1966)	Modified method	MRD/ 1 ml/ of method
C.0.1	1/2	1/4	1/2	20 MRD/1 ml.
C.0.2	non toxegenic	-	-	-
C.0.3	1/2	1/4	1/4	40 MRD/1 ml.
C.0.4	1/2	1/2	1/2	20 MRD/1 ml.
C.0.5	non toxegenic	-	-	-
C.0.6	1/2	1/4	1/2	20 MRD/1 ml.
C.0.7	1/4	1/4	1/2	20 MRD/1 ml.
C.0.8	1/4	1/4	1/16	160 MRD/1 ml.
C.0.9	1/2	1/4	1/64	640 MRD/1 ml.
C.0.10	1/2	1/4	1/8	80 MRD/1 ml.

Abbreviations:

MRD - Minimum Reacting Dose.

DISCUSSION

Ten strains of C.ovis were collected from different localities of Egypt and were examined for toxin production by using three methods. The

level of exotoxin produced was titrated by means of rabbit skin sensitivity test.

In this work 8 strains were found to be toxinogenic while two strains (C.o. 2 and C.o.5) were non toxinogenic. These results come in accordance with the finding of WOODRUFF and OXER (1929), REIMANN (1930) and LOVELL and ZAKI (1966) who reported that out of 75 isolates of C. ovis recovered from sheep,cattle, horse and deer,73 strains were toxinogenic and 2 non-toxinogenic. On contrary, CARNE (1940) and DOTY et al. (1964) reported that all examined C.ovis strains produced exotoxin.

For measuring the potency of C.ovis toxins in this investigation, the minimum reaction dose in rabbit skin test was applied. DOTY et al. (1964) considered the minimal reacting dose as the highest dilution of toxic broth which when injected intradermally into rabbit caused a reaction in the form of small oedematous swelling (1.5 cm swelling) after 48 hours from injection. However determination of standard unit for measuring the potency of C.ovis toxin is up till now not constant and few workers who had investigated such as DOTY et al. (1964) used the minimum reacting dose (MRD/ml), while LOVELL and ZAKI (1966) used the minimal lethal dose (MLD) which was the least amount of toxin capable of killing all the inoculated mice within 4 days. In this work the use of rabbit skin sensitivity test was more reliable, economic and safe the time of the experiments.

The present study showed that the methods used for toxin production gave variable results as the two methods of DOTY et al.(1964) and LOVELL and ZAKI (1966) methods gave relatively less potent toxins where the MRD/ml of the produced toxins ranged from 20-40. On the other hand, the toxins produced by the modified methods had minimal reacting dose ranged from 20 by four strains to 640 by strain C.o. 9 (Table 2). Therefore it can be suggested that the use of veal infusion agar and modified digest media is more satisfactory and reliable for the production of potent toxin of C.ovis.

Also it was noticed that the prepared toxin must be used within one month where the original toxicity become reduced, when the toxin was not covered with any reducing agent. This suggestion agreed with addition of a reducing agent was necessery to keep the original toxicity.

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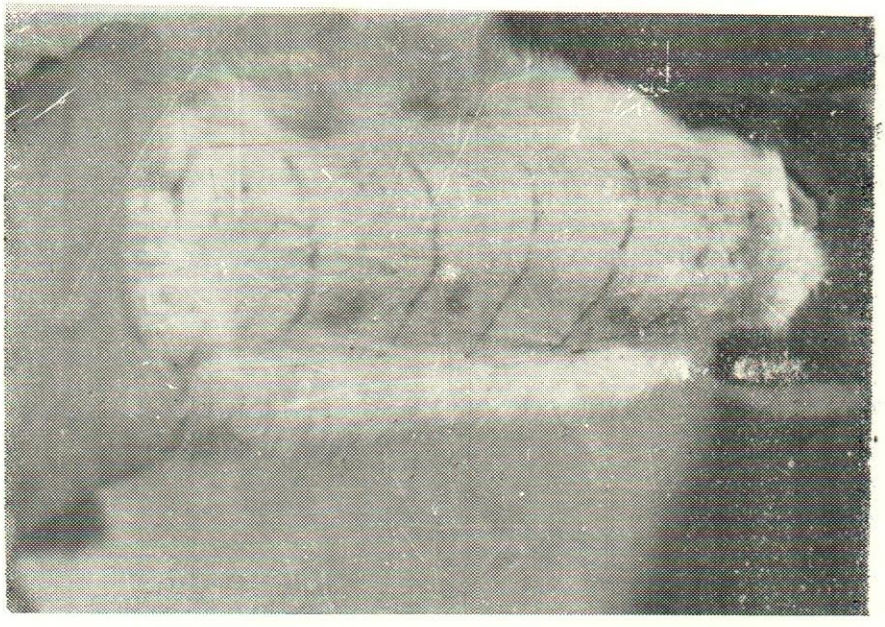

(Fig. 1)

Determination of minimum reacting dose of C. ovis (C.O.G.) by means of rabbit skin sensitivity serum neutralization test (Doty et al.).