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BACTERIA ISOLATED FROM EGYPTIAN SNAKES VENOM USED FOR PREPARATION OF ANTISERA

Okela, M.A.[a], Khalil, S.A.[a], Abir A. Elfiky[b], Abd El-Ghafar, M.M[c]

[a] Dept. of Microbiology, Fac. Vet. Med., Alex. Univ., [b] ANDI center of excellence in antivenom research, Holding Company for biological products and vaccines (VACSERA), [c] Holding Company for biological products and vaccines (VACSERA).

A B S T R A C T

The causes of the inflammatory reactions associated with immunization of horses with Egyptian Cobra venom for production of cobra anti-venom was studied. Lophylized snake venom collected from Cobra snakes were subjected to microbiological examinations, including culture on microbiological media and confirmation to the isolated bacteria biochemically and API analytical profile index 20NE. The result revealed presence of *Vibrio vulnifacus* bacteria in Egyptian cobra venom while there is no any bacterial or fungal contaminant in spitting cobra venom. So, it is recommended to take several considerations during immunization of horses by Egyptian cobra venom for production of anti-venom.

KEY WORDS: Bacteria, Snake venom, Vibrio vulnifacus

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1. I N T R O D U C T I O N

Preparation of snake anti-venom includes administration of the venom to a suitable animal -mostly horses- and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal. During such procedure, the recipient animal may suffer different types of ill-health signs [1]. The severity and duration of the observed clinical signs depend on the nature, amount and site of the injected venoms [2] Microbiological examination of some snakes families including elapids revealed presence of high diversity in isolated bacteria including Gram-negative bacilli, Gram-positive bacilli and Gram-positive cocci in the oral cavity with or without fangs. These bacteria also accused to cause abscess formation at the bite site and pathogenic process in snakes that host this microorganisms [3]. Immunization of horses with Egyptian Cobra venom for production of cobra antivenom associated with different local reaction as inflammation and tissue destruction [4, 5] manifested by abscess in the injection site as a local reaction and systemic reactions [3].

2. MATERIAL AND METHODS

In this experiment bacteria was isolated collected from snakes venom (Egyptian Cobra, and Spitting Cobra) from VACSERA, venomous animal, and toxin center) and identified using different methods for identification including gram stain, Biochemical reaction tests (Indole test, Oxidase test, Urease test and Citrate utilization test), and API analytical profile index 20NE (Biomerieux, Inc., Hazelwood, MO). Different types of media were used in this work for isolation, identification and propagation of bacteria isolated from snake venom. Media were prepared according to lab M, England,

Chemicals and reagent test kits prepared according to **[6].** All the snakes used in this study were obtained from the municipality of VACSERA and were maintained in captivity at the animal house of Helwan farm. Venomous Animals that represented each one of two snake species were chosen for oral cavity swabbing. The two species were: *Naja haje, Naja nigricollis.* The snakes were milked under sterile condition from adult snakes in special way using a beaker covered with a plastic sheet. After collection physical examination of the collected venom was done, then, the venom was kept in special vials each contained 1 ml, and kept at (20ºC) for 24 hours and then lyophilized in a Lyopholizer. Just prior to inoculation into the recipient animal, the venom was dissolved in sterile saline/dose of the venom, and microbial examination was done. For Microbiological examination of venom ten collected samples from each snake species were used for the microbiological study. Bacteria isolated from snakes, classified according to genus and species according to morphological, cultural and biochemical studies according to [7]. For primary isolation of the bacteria The samples were dissolve in sterile saline and seeded into plates of nutrient, macConky, blood agar, and Sabouraud agar (Difco, USA) and incubated at 36°C, for 24 hours, in the Microbiology Laboratory, R&D sector, VACSERA. Secondary, purification of isolated bacteria by picked up isolated growing colonies and subculture it (The primary isolation) on the same seed media for more purification. The bacteria obtained isolates were inoculated in semisolid agar for preservation and motility test. Also, the obtained bacterial isolates were subculture on slope agar and use as a stock culture for further inoculated in semisolid agar for Identification. Identification of recovered isolates: including, morphological examination using dry heat fixed smears which prepared and stained with gram´s stain and examined for morphological characteristic and staining reaction under the microscope used oil immersion lens. Culture characteristic: Including morphological characterization of the growth colony (shape, color, texture, appearance, pigmentation and haemolysis). Biochemical identification: The isolates were subjected for the following biochemical examination (Indole, Oxidase, Urease, and citrate utelization test, catalase, and sugar fermentation test). Mean while, the isolates were retested biochemically using API analytical profile index 20NE (biomerieux, INC., Hazelwood, MO) at the bacteriological lab of Ministery of health laboratories as a stander lab to confirm our results of identified bacteria isolates. The API 20 NE strip consists of 20 microtubes containing dehydrated media and substrates. The covential tests are inoculated with saline bacterial suspention which consist the media. Colour change is observed either spontaneous or revealed by addition of agents differ according to tests need. Identification can be obtained using the analytical profile index by coding the observed reactions into numerical profile on result sheet, using the identification software by manually entering the 7-digits numerical profile to identify the target tested bacteria.

3. RESULTS AND DISCUSSION

Inoculation of Egyptian cobra venom in nutrient broth, nutrient agar, MacConky agar, blood agar, and Sabouraud agar result in 100% growth in cultivated tubes manifested by turbidity in the inoculated broth tubes and presence of round creamy colony dispersed all over the inoculated plate beside change of media colour from pink to yellow colour of MacConky agar plate. While no growth in Sabouraud agar cultivated plates (0%). On contrast, the inoculation of spitting cobra venom in the previous types of media result in free from any growth (0%) compared with the control non inoculated plates (Table 1, 2).

these result agree with [8], who showed strong relationship between microorganisms present in abscesses or in patient lesions and the ones from snake oral cavities, and [3] who detected presence of higher diversity of isolated bacteria from the oral cavity of Elapidae. In this study the isolated bacteria cause change color of the macCkonky media from pink to colorless this indicate that this bacteria is non lactose fermenter, also the bacterial growth is evidenced by a follicular deposit on top of the blood layer and no hemolysis on blood agar this indicate that our isolate may be gama hemolytic bacteria [7]. The observed results were presence of round creamy smooth opaque non pigmented, non lactose fermentor, and non haemolytic colonies this indicate that the tested isolated bacteria may be involved in enterobacteriacae or non enterobacteriacae families, this result agree with [7].

Table 1 Bacteria isolated from the cobra venom

Type of Media	Control		Result of bacterial growth		
	n	Growth (%)	n	Bacterial Growth	Growth (%)
Nutrient broth	10	100	10	10	100.00
Nutrient agar	10	100	10	10	100.00
MacConkey agar	10	100	10	10	100.00
Blood agar	10	100	10	10	100.00
Sabouraud"s agar	10	100	10	0	00.00

n: indicated number of samples

Table 2 Result of culturing of spitting venom on seed media

Type of Media	n	Growth (%)
Nutrient broth	10	100%
Nutrient agar	10	100%
macConkey agar	10	100%
Blood agar	10	100%
Sabouraud's dextrose agar	10	100%

Isolated colony were cultivated on Motility-Indole-Ornithine (MIO) media, resulted in presence of diffuse growth in both side of the stabbing line, and yellow ring on the surface of media beside change in the media color to transparent color especially at the bottom of the tube. This indicates that the isolate is motile bacteria, negative indole, and ornithin positive. This result revealed that this isolate may be non enerobacteriacae possible to be vibrio species according to [7]. Biochemical examination of the isolate including Indole, Oxidase, Urease, and Citrate Utilization test, and glucose fermentation test result in yellow ring by Indole test, violet color by oxidase test, yellow colour by urease test, blue color by citrate utilization test, yellow color by glucose fermentation test indicate that the isolate is included in non enterobacteriacae family due to positive result of oxidase test (Table, 5) which agree with [7].

Table 3: Microscopical examination of the isolate by Gram stain

Colony isolated from	Gram stain	Shape
Nutrient agar	Negative	Curved rod With slight filament.
MacConkey agar	Negative	Curved rod With slight filament.
Blood agar	Negative	Curved rod With slight filament.

To confirm our result the isolates were retested biochemically using API analytical profile index 20E, and 20NE. By using API 20 E analytical profile test we found that the isolate cause colour reaction, this color result is obtained using the analytical profile index by coding the observed reactions into numerical profile on result sheet, using the identification software by manually entering the 7-digits numerical profile via key board, the target

tested bacteria is identified using the 7-digits are (0106707) for API 20E, the result entered give no ID this means that the isolate not belongs to Enterobacteriacae family and may belong to non Enterobacteriacae family, This agree with biomerieux, INC., Hazelwood, MO.

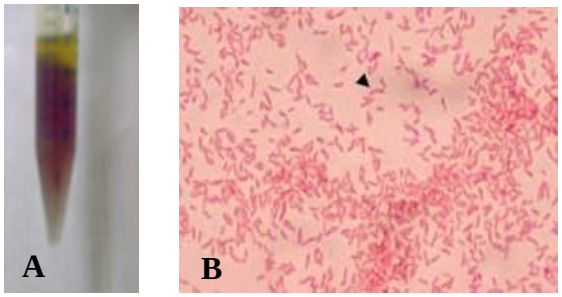

Photo 1 Isolated bacteria from snake venum. To the left (A) isolated colony on MIO media. And to the right (B) isolated bacteria under oil immersion lens of microscope.

Table 4 Culture characters of the purified bacterial colonies

	Source of isolated colony		
Properties	Nutrient agar	MacConkey agar	Blood agar
Shape	round	round	round
Color	Creamy	Creamy non lactose fermenter	Creamy
Texture	smooth	smooth	smooth
Appearance	Opaque	Opaque	Opaque
Pigmentation	non	non	Non
Haemolysis	-----	-----	Non hemolytic

Table 5 Cultivation of colony isolated from cobra venom on MIO Media (Motility-Indole-Ornithine)

Test	Result
Motility	Motile
Indole	Indole negative
Ornithine	Ornithine positive

Table 6 Biochemical reactions of bacteria isolated from cobra venom:

Test	n	Reaction	%
Indole	10	Negative yellow ring	100%
Citrate	10	Positive blue colour	100%
Urease	10	Negative yellow colour	100%
Oxidase	10	Positive violet colour	100%
Glucose fermentation	10	Positive yellow colour	100%

n: indicated number of samples

4. CONCLUSION

It could be concluded that Egyptian elapids venom specially E.cobra contain gram negative curved motile rod bacteria, that bacteria when characterised biochemically revealed (reactions). API test was applied to confirm our result, and represent presence of bacteria involved to non enterobacteriacae family by both APIE and API NE, using the analytical profile index by coding the observed reactions into numerical profile on result sheet, using the identification software, the target tested bacteria is identified to be [1]. In contrast Spitting cobra venom was sterile that give no microbiological growth either bacterial or fungal.

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