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# Production of enterotoxins by strains of *Staphylococcus aureus* isolated from camels in Nigeria

by A. A. ADESIYUN

Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.

**RÉSUMÉ**

ADESIYUN (A. A.). — Production d'entérotoxines par des souches de *Staphylococcus aureus,* isolées de dromadaires au Nigeria. *Rev. Elev. Méd. vét. Pays trop.,* 1985, **38** (4) : 423-427.

A partir d'écouvillonnages effectués sur la partie antérieure des naseaux et dans le rectum de 175 dromadaires abattus à Kano (Nigeria), on a effectué des cultures de *Staphylococcus aureus* sur milieu gélosé de Baird-Parker (BPA). La production d'entérotoxines par les souches isolées a été déterminée au moyen de la technique d'immunodiffusion en gélose.

Sur les 210 souches de *S. aureus* testées, 39 souches ont produit des entérotoxines staphylococciques de type A (SEA), B (SEB), C (SEC), D (SED) ou E (SEE). L'entérotoxine la plus fréquemment produite était de type SEA, avec 12 (5,7 p. 100) souches productives, représentant 30,8 p. 100 du total des souches entérotoxinogènes. Les autres souches se répartissaient comme suit : 10 (4,8 p. 100) pour le type SED ; 9 (4,3 p. 100) pour le type SEB ; 8 (3,8 p. 100) pour le type SEE ; 4 (1,9 p. 100) pour le type SEC.

27 (22,3 p. 100) des 121 souches isolées à partir du rectum ainsi que 12 (13,5 p. 100) des 89 souches isolées des narines étaient entérotoxinogènes.

De nombreuses entérotoxines étaient de type AB, AC, AE et DE, chacune étant élaborée par une souche de *S. aureus.* Le pouvoir entérotoxinogène des souches isolées était plus élevé parmi les souches productives de coagulase, de thermonuclease et d'alpha hémolysine. La prédominance de la production de SEA par les *Staphylococcus* isolés de dromadaires du Nigeria semble indiquer que ce type pourrait être responsable de la plupart des intoxications staphylococciques, consécutives à la consommation des produits et de la viande de dromadaire au Nord Nigeria.

*Mots clés :* Dromadaire - *Staphylococcus aureus* - Entérotoxines - Nigeria.

**SUMMARY**

ADESIYUN (A. A.). — Production of enterotoxins by strains of *Staphylococcus aureus* isolated from camels in Nigeria. *Rev. Elev. Med. vét. Pays trop.,* 1985, **38** (4) : 423-427.

Swabs of the anterior nares and recta of 175 camels slaughtered at Kano abattoir, Nigeria, were cultured for *Staphylococcus aureus* on Baird-Parker agar (BPA). Enterotoxin production by isolates was determined by the double gel diffusion technique. Thirty-nine (18.6 p. 100) of 210 *S. aureus* strains tested produced staphylococcal enterotoxins A (SEA), B (SEB), C (SEC), D (SED), or E (SEE). The most frequently elaborated enterotoxin was SEA with 12 (5,7 p. 100) strains being producers and representing 30.8 p. 100 of all enterotoxigenic strains. Ten (4.8 p. 100) strains produced SED while 9 (4.3 p. 100), 8 (3.8 p 100), and 4 (1.9 p. 100) strains secreted SEB, SEE and SEC, respectively. Twenty-seven (22.3 p. 100) of 121 isolates from recta and 12 (13.5 p. 100) of 89 isolates from anterior nares were enterotoxigenic.

Multiple enterotoxins produced were types AB, AC, AE and DE which were each elaborated by a strain of *S. aureus.* Enterotoxigenicity of isolates was highest amongst coagulase, thermonuclease and alpha hemolysin producers.

The predominance of SEA production amongst isolates from camels in Nigeria suggests that it may be responsible for most staphylococcal intoxication resulting from consumption of camel meat and products in Northern Nigeria.

*Key words :* Dromedary camel - *Staphylococcus aureus* - Enterotoxins - Nigeria.

## INTRODUCTION

Certain strains of *Staphylococcus aureus* are known to produce enterotoxins which are responsible for staphylococcal foodpoisoning outbreaks in several countries (6, 21, 23). Enterotoxigenic staphylococci have also been isolated from several animals, cattle, horses, goats, sheep, swine, rats, dogs and chickens, (11, 14, 15). To date, however, information is

not available on the enterotoxigenicity and other properties of strains of *S. aureus* from camels.

The F.A.O. (22) estimates that over 1,700 single-humped tropical camels (*Camelus dromedarius*) of a total population of 17,000 are slaughtered annually in Northern Nigeria. Camel meat is rapidly becoming a popular source of animal protein for human population. In addition to cooking camel meat in households, it is also roasted into a delicacy locally called « suya », which is a ready-to-eat product. In a recent study, a number of ready-to-eat products of beef (cattle) originated in Nigeria were found to be contaminated by enterotoxigenic staphylococci (3).

This study was conducted to determine the ability of *S. aureus* strains from camels for slaughter at an abattoir in Nigeria to produce enterotoxins.

## MATERIELS AND METHODS

### Source of sample

Between February and May 1984, a total of 175 apparently healthy single-humped camels brought to the Kano abattoir, Nigeria, for slaughter were randomly selected and sampled. For each camel, swabs of the anterior nares and rectum were obtained.

### Sample collection

Two sterile moist swabs were used for each camel. One was applied to the rectum and the other to the anterior nares. Each sample was immediately put in a sterile test tube.

### Isolation of staphylococci

The procedure earlier described by ADESIYUN and USMAN (4) was used.

### Fermentation of mannitol by isolates

The ability of isolates to ferment mannitol was determined in oxidative-fermentation tubes as modified by the Subcommittee on Taxonomy of Staplylococci and Micrococci (19). Acid production in 5 days of incubation was considered as a positive reaction.

### Coagulase detection

The coagulase test was performed on a 24 h growth at 37 °C in Brain heart infusion (BHI) broth using freshly prepared human plasma diluted 1/4 with sterile saline. The procedure of BAER *et al.* (5) and test interpretation protocol of SPERBER and TATINI (18) were used.

### Thermonuclease detection

Production of thermonuclease was detected by the test procedure of LACHICA *et al.* (12).

### Hemolysin detection

Production of alpha, gamma and beta hemolysins was determined with washed sheep erythrocytes added to blood agar to obtain a final concentration of 3 p. 100. Interpretation of hemolysin was made after 48 h at 37 °C as described by ELEK and LEVY (9).

### Enterotoxin production and detection

The cellophane-over-agar method of ROBBINS *et al.* (17) was used for growing isolates for enterotoxin production. Enterotoxin was detected by the double gel diffusion (microslide) technique of CASMAN and BENNETT (7) using standard antisera A to E.

## RESULTS

The incidence of enterotoxin production by *Staphylococcus aureus* strains isolated from camels in Nigeria is shown in table n° I. Thirty-

TABLE N° I - Incidence of enterotoxin production by *Staphylococcus aureus* strains isolated from camels

| Source | Nb. of strains tested | Nb. (p.100) of strains enterotoxigenic | Nb. (p.100) of strains which produced enterotoxins | | | | |
|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E |
| Anterior nares | 89 | 12 (13.5) | 5 (5.6) | 2 (2.2) | 1 (1.1) | 3 (3.4) | 3 (3.4) |
| Rectum | 121 | 27 (22.3) | 7 (5.8) | 7 (5.8) | 3 (2.5) | 7 (5.8) | 5 (4.1) |
| Total | 210 | 39 (18.6) | 12 (5.7) | 9 (4.3) | 4 (1.9) | 10 (4.8) | 8 (3.8) |

nine (18.6 p. 100) of 210 isolates tested were enterotoxigenic. Twelve (13.5 p. 100) of 89 isolates from the anterior nares were entorotoxigenic compared with 27 (22.3 p. 100) of 121 isolates from the recta. Staphylococcal enterotoxin A (SEA) was most frequently produced, 12 (5.7 p. 100) followed by SED, 10 (4.8 p. 100) and SEB, 9 (4.3 p. 100). Staphylococcal enterotoxin E (SEE) and SEC were produced by 8 (3.8 p. 100) and 4 (1.9 p. 100) strains, respectively.

The distribution of multiple enterotoxins produced by *S. aureus* strains in shown in table n° II. The only 4 multiple enterotoxins detected, AB, AC, AE and DE were elaborated by 1 strain of *S. aureus* each.

Table n° III shows the relationship between mannitol fermentation, coagulase, thermonuclease, hemolysin and enterotoxin production by staphylococcal isolates. All 39 enterotoxigenic strains fermented mannitol and produced coagulase. None of the 3 strains that failed to ferment mannitol was enterotoxigenic. Three (14.3 p. 100) of 21 thermonuclease-negative strains were enterotoxigenic compared with 36 (19.0 p. 100) of 189 thermonuclease-positive found enterotoxigenic. Of the 210 isolates, 72 (34.3 p. 100) produced alpha hemolysin, while 74 (35.2 p. 100) elaborated beta hemolysin while 64 (30.5 p. 100) had gamma hemolytic pattern. The incidence of enterotoxin production was highest amongst alpha hemolysin producers, 22.2 p. 100 and least amongst beta hemolysin producers, 13.5 p. 100. Thirteen (20.3 p. 100) of 64 isolates with gamma hemolytic pattern were enterotoxigenic.

## DISCUSSION

This is considered the first report on the enterotoxigenicity of *S. aureus* strains isolated from the single-humped tropical camel. The finding that 18.6 p. 100 of the isolates tested were enterotoxigenic is of importance from the viewpoint of food hygiene. Since the samples were obtained from camels brought for slaughter,

TABLE N° II - Distribution of multiple enterotoxins produced by *Staphylococcus aureus* strains isolated from camels

| Source | Nb. of strains which produced enterotoxins A-E | Nb. of strains of *S. aureus* which produced enterotoxins | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | A | AB | AC | AE | B | C | D | DE | E |
| Anterior nares | | 3 | 1 | 1 | - | 1 | - | 3 | - | 3 |
| Rectum | | 6 | - | - | 1 | 7 | 3 | 6 | 1 | 3 |
| Total | | 9 | 1 | 1 | 1 | 8 | 3 | 9 | 1 | 6 |

TABLE N°III - Relationship between mannitol fermentation, coagulase, thermonuclease, hemolysin and enterotoxin production by staphylococcal isolates

| | Number of isolates | Number of isolates enterotoxigenic | Percentage of isolates enterotoxigenic |
|---|---|---|---|
| Mannitol fermentation | | | |
| Positive | 207 (98.6) | 39 | 18.8 |
| Negative | 3 (1.4) | 0 | 0.0 |
| Coagulase production | | | |
| Positive | 210 (100.0) | 39 | 18.6 |
| Negative | 0 (0.0) | 0 | 0.0 |
| Thermonuclease production | | | |
| Positive | 189 (90.0) | 36 | 19.0 |
| Negative | 21 (10.0) | 3 | 14.3 |
| Hemolysin production | | | |
| Alpha | 72 (34.3) | 16 | 22.2 |
| Beta | 74 (35.2) | 10 | 13.5 |
| Gamma | 64 (30.5) | 13 | 20.3 |

the possibility of contamination of meat and meat products originating from camels cannot be ruled out. Camels are a significant source of animal protein for most of human population in Northern Nigeria. The F.A.O. (22) had earlier reported that about a tenth of the single-humped tropical camels are slaughtered annually in Northern Nigeria.

The incidence (18.6 p. 100) of enterotoxin production amongst *S. aureus* strains originating from camels in the present study is comparable to those reported for other animal species in Nigeria. The enterotoxigenicity of *S. aureus* isolates from cattle, sheep, goats and dogs were found to be 12.0 p. 100, 19.0 p. 100, 23.5 p. 100 and 20.9 p. 100, respectively by ADEKEYE (1). Recently ADESIYUN and USMAN (4) found 16.8 p. 100 of *S. aureus* strains from dogs around Zaria, Nigeria, to be enterotoxigenic after sampling more dogs than reported in an earlier study.

It was of interest to observe that all 5 presently known staphylococcal enterotoxins (SEA, SEB, SEC, SED and SEE) were produced by isolates from camels. HAJEK and MARSALEK (11) had earlier stated that production of SEA, SEB and SEC were rare in animals. Other studies in this environment have demonstrated that these 3 enterotoxin types are commonly encountered in *S. aureus* isolates of animal origin (1,4).

The predominance of SEA production (5.7 p. 100) by isolates from camels is in agreement with reports on the enterotoxigenicity of animal isolates of *S. aureus* in Nigeria (1,4) with the exception of dogs where SEC predominates (4). SEA and SED are known to be most frequently implicated in staphylococcal foodborne intoxication in other countries where reporting of foodborne diseases is practised (6, 16, 23). ADESIYUN (3) also found SEA and SED to be most commonly secreted by *S. aureus* strains isolated from ready-to-eat food products in Nigeria.

That the 210 staphylococcal isolates were all coagulase-positive is an indication that these are indeed *S. aureus*. Coagulase production is a major criterion used in the classification of staphylococci as *S. aureus* (6). Three (1.4 p. 100) of these isolates failed to utilize mannitol anaerobically, despite the fact that fermentation of glucose and mannitol is a property of *S. aureus* (6). DEVRIESE and OEDING (8) had, however, reported that some *S. aureus* strains from animals such as pigeons and dogs had weak ability of failed to ferment mannitol. It is presently not known whether *S. aureus* from camels also display this characteristic commonly.

The finding that 90.0 p. 100 of the isolates were thermonuclease-positive agrees with an earlier report in dogs in Nigeria where 82.2 p. 100 of isolated tested produced thermonuclease (4). Yet the present results are at variance with those of ADEKEYE (2). The author recently reported a low incidence of thermonuclease production by *S. aureus* isolates from sheep (14.2 p. 100), goats (25.0 p. 100) and cattle (34.1 p. 100). It was even suggested that the thermonuclease test may not be very useful for confirming the *S. aureus* isolates of animal origin. Although the present study agrees, in part, with the report of TATINI (20) that almost all coagulase-positive strains of *S. aureus* are thermonuclease-positive suggesting that the thermonuclease test should replace the coagulase test in confirming *S. aureus* isolates. This is because of the numerous advantages of the former over the latter test. However, data obtained in the present study disagree with the suggestion that thermonuclease production could be used as an index of enterotoxigenicity (20). This is because the incidence of enterotoxin production amongst thermonuclease-positive (19.0 p. 100) and thermonuclease-negative (14.3 p. 100) isolates were similar. It is also pertinent to mention that a close relationship between coagulase, thermonuclease and enterotoxin production was earlier detected by LACHICA *et al.* (13). All these findings stress a need to further determine this relationship with due consideration to the sources of staphylococcal isolates.

The incidence of alpha hemolysin (34.3 p. 100) and beta hemolysin (35.2 p. 100) production by isolates were similar. This is surprising because it is known that animal biotypes of staphylococci produce beta hemolysin predominantly while human biotypes mainly elaborate alpha hemolysin (9, 10). This finding can be explained, in part, by earlier evidence of interchange of staphylococcal biotypes between animals and human beings in Nigeria (1,3). A similar occurrence may be spectulated between camels and their human handlers.

In conclusion, it appears that SEA may be responsible for most of staphylococcal intoxication resulting from consumption of camel meat and products in some parts of Northern Nigeria.

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## RESUMEN

ADESIYUN (A. A.). — Producción de enterotoxinas por cepas de *Staphylococcus aureus,* aisladas de dromedarios en Nigeria. *Rev. Elev. Méd. vét. Pays trop.,* 1985, **38** (4) : 423-427.

A partir de escobillonares efectuados en la parte anterior de las ventanas de la nariz y en el recto de 175 dromedarios matados en Kano (Nigeria), se efectuaron cultivos de *Staphylococcus aureus* sobre medio de gelosa de Baird-Parker. Se determinó la producción de enterotoxinas por las cepas aisladas mediante la técnica de inmunodifusión en gelosa.

De las 210 cepas de *S. aureus* observadas, 39 cepas producieron enterotoxinas de estafilococos de tipo A (SEA), B (SEB), C (SEC), D (SED), o E (SEE).

Era de tipo SEA la enterotoxina más frecuentemente producida, con 12 (5,7 p. 100) cepas productivas, representando 30,8 p. 100 del total de las cepas enterotoxinogenas. Entre demás cepas, 10 (4,8 p. 100) eran de tipo SED ; 9 (4,3 p. 100) de tipo SEB ; 8 (3,8 p. 100) de tipo SEE ; 4 (1,9 p. 100) de tipo SEC. 27 (22,3 p. 100) de las 121 cepas aisladas a partir del recto así como 12 (13,5 p. 100) de las 89 cepas aisladas de las partes anteriores de las ventanas de la nariz eran enterotoxinogenas.

Numerosas enterotoxinas eran de tipo AB, AC, AE y DE, cada una proviniendo de una cepa de *S. aureus.*

El poder enterotoxinogeno de las cepas aisladas era más elevado entre las cepas productivas de coagulase, de termonuclease y de alfa hemolisina.

El predominio de la producción de SEA por *Staphylococcus* aislados de dromedarios del Nigeria parece indicar que este tipo podría ser causa de la mayoría de las intoxicaciones debidas al consumo de los productos y de la carne del dromedario en el norte de Nigeria.

*Palabras claves :* Dromedarios - *Staphylococcus aureus* - Enterotoxina - Nigeria.

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