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Frequency of isolation of enterotoxigenic staphylococci from milk of nursing mothers in Kaduna, Nigeria

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SUMMARY

Milk samples from 251 nursing mothers were screened for enterotoxigenic staphylococci. The incidence of staphylococci in milk samples was 71·3%. Two hundred and sixteen strains were isolated from 179 mothers. Eighty-six (39·8%) of the 216 strains were found to be toxigenic. Enterotoxin type A (SEA) predominated, with 41 strains (19·0%) elaborating it. Twenty-one strains (9·7%) produced enterotoxin B (SEB) while only eight (3·7%) produced enterotoxin C (SEC). Ten strains (4·6%) produced all three types. Enterotoxigenic strains usually produced coagulase, thermonuclease and alpha haemolysin.

In this series breast-feeding alone was more common than combined breast and bottle feeding, especially among mothers less than 30 years old. The incidence of reported infantile diarrhoea decreased with increasing age of the mother. Of 16 babies with diarrhoea, 10 (62·5%) had mothers whose milk yielded staphylococci. Six of these were toxigenic. Although no direct relationship between enterotoxigenic staphylococci in the milk of nursing mothers and infantile diarrhoea could be demonstrated, these findings reveal a potential health risk to these infants.

INTRODUCTION

Bovine milk and milk products have often been incriminated as vehicles for staphylococcal food poisoning (CDC, 1976; Todd, 1978, 1981). Incidents have been reported in which staphylococci have grown and multiplied in the gut with release of enterotoxin (Casman, 1965; Kienitze, 1966; Hallander & Korlof, 1967).

Enterotoxigenic staphylococci have been isolated from pus in cases of human mastitis (Mochmann *et al.* 1979) although in these cases it was thought that the mastitis was sometimes caused by strains of staphylococci carried in the babies' noses rather than the reverse, and enterotoxigenic strains have been isolated from breast milk of mothers whose children were suffering from diarrhoea (Burbianka, 1971; Burbianka & Dluzniewska, 1971). Zak, Jeljaszewicz & Stochmal (1973) found 75·6% of staphylococcal strains from human cases of diarrhoea in Poland to be

toxigenic. All these findings suggest a possible *in vivo* role for staphylococci as a cause of diarrhoea in addition to the more usually recognised staphylococcal food poisoning. Infants, with relatively unestablished gastro-intestinal microflora, probably constitute a special high risk sector of the population, especially if exposed to enterotoxigenic staphylococci in breast milk.

In view of a recent campaign by the World Health Organisation to promote breast feeding over bottle feeding, especially in developing countries such as Nigeria, where poor sanitation and inadequate medical facilities are often found, we decided to screen breast milk of apparently healthy mothers for enterotoxigenic staphylococci and to try to relate this to the occurrence of infantile diarrhoea.

MATERIALS AND METHODS

Population sampled

A single milk sample was obtained from each of 251 nursing mothers attending the weekly postnatal clinic of Ahmadu Bello University Teaching Hospital (ABUTH) between October and December 1981. On any sampling day about 30 mothers were selected at random from those attending the clinic. No mother was sampled more than once. A prepared questionnaire was used to find out the ages of mother and infant, the number of previous births, any history of mastitis, details of the infant's feeding regime and any incident of infantile diarrhoea.

Sample collection and culture

Approximately 7 ml of breast milk was expressed directly into a sterile 10 ml sample bottle using aseptic precautions and with the help of nursing attendants. The samples were delivered to the laboratory as a batch later the same day.

After thorough shaking approximately 0·01 ml volumes were streaked with a standard wire loop on plates of Baird-Parker Agar (BPA). The plates were incubated at 37 °C for 48 h. Colonies resembling staphylococci were subcultured into Brain Heart Infusion broth (BHI) and incubated overnight at 37 °C. They were then stored on nutrient agar slants at 4 °C prior to formal identification by standard methods (Cowan & Steel, 1965).

Coagulase production

The coagulase test was performed on each isolate, using fresh human plasma, in test-tubes, according to the recommendations of the Subcommittee on Taxonomy of Staphylococci and Micrococci (1965).

Haemolysin production

Haemolysin production by staphylococcal isolates was determined on washed sheep red blood cells incorporated in agar plates. Interpretation of haemolytic pattern was as described by Elek & Levy (1950).

Thermonuclease production

Production of thermostable deoxyribonuclease by the isolates was determined by the methods of Lachica, Genigeorgis & Hoeprich (1971). Toluidine blue *O*-deoxyribonucleic acid agar, 13 ml, was poured into a petri dish (15 × 100 mm),

Table 1. *Relationship between age of mother, mean number of births, carrier status of enterotoxigenic staphylococci in milk, type of feeding and incidence of diarrhoea in infants*

Age range (years)	Mothers sampled		Mean no. of births per mother	Feeding pattern			Samples yielding staphylococci				Infants with diarrhoea	
	Number	%		Breast only	%	Breast and bottle	Total	%	entero toxo-genic	%	Number	%
16–20	57	22·7	1·9	38	66·7	19	41	71·9	21	51·2	6*	10·5
21–25	103	41·0	3·4	73	70·9	30	76	73·8	33	43·4	9†	8·7
26–30	41	16·3	5·2	23	56·1	18	24	58·5	9	37·5	1‡	2·4
> 30	50	19·9	5·4	36	72·0	14	38	76·0	23	60·5	0	0·0
Total	251	100·0	3·7	170	67·7	81	179	71·3	86	48·0	16	6·4

* Six infants: milk from two mothers contained enterotoxigenic staphylococci, from two contained non-enterotoxigenic staphylococci and two were free from staphylococci.

† Nine infants: three enterotoxigenic, two non-enterotoxigenic, four free from staphylococci.

‡ This infant's mother yielded an enterotoxigenic strain.

Table 2. *Serological types of enterotoxins* produced by strains of staphylococci isolated from milk of nursing mothers*

Type of enterotoxin	Number of strains	%
A	41	19·0
AB	10	4·6
ABC	2	0·9
AC	4	1·9
B	21	9·7
C	8	3·7
None	130	60·2
Total	216	100·0

* Isolates were tested for production of enterotoxins A, B and C.

20 wells were cut into the agar with the tip of a 2 ml plastic syringe. To each well was added a drop of an overnight Brain Heart Infusion broth culture of an isolate which had been steamed for 15 min at 100°C and cooled. Each plate was incubated at 37°C in humid conditions overnight. Organisms with bright pink zones were regarded as thermostable deoxyribonuclease-positive.

Growth and enterotoxin production

The cellophane-over-agar method described by Robbins, Guld & Bergdoll (1974) was employed. However, in this investigation, cellophane discs were cut from dialysis tubing-visking size 16–$2\frac{2}{3}$ in. (Medicell International Ltd, London). Harvested cells were spun down for 15 min at 7000 rpm. Production of enterotoxins A, B and C was assayed in culture supernatant by the double gel diffusion technique of Casman & Bennett (1965). Prior to reading of the slides at an oblique angle against a fluorescent desk lamp, they were stained with Thiazine Red-R (American Hoechst Corp., USA) for 10 min. Standard antigens and antisera used were kindly provided by Professor S. R. Tatini of the University of Minnesota, USA.

RESULTS

The age distribution, mean parity and feeding patterns of the 251 nursing mothers are shown in Table 1. One hundred and three (41·0 %) of the mothers were between 21 and 25 years old. Mean parity increased with age, as expected. Breast feeding alone was more common than breast plus bottle feeding in all age groups, and showed no correlation with maternal age.

The age of the babies at time of sampling varied from 3 to 40 days, with a mean age of 20·9 days. Sixteen infants were reported to have had some diarrhoea. The incidence of diarrhoea was unrelated to the age of the baby but markedly decreased with increasing maternal age (Table 1). No mother over 30 years old reported her baby to have had diarrhoea.

Staphylococci were isolated from 179 of the milk samples; 37 samples yielded two different colonial types, so that a total of 216 strains of staphylococci were eventually examined. Eighty-six strains proved to be enterotoxigenic, 57 producing staphylococcal enterotoxin SEA either alone (41 strains) or together with SEB

Table 3. *Relationship between coagulase, thermonuclease, hemolysin and enterotoxin production by isolates**

	Coagulase		Thermonuclease		Hemolysin		
	Positive	Negative	Positive	Negative	Alpha	Beta	Gamma
No. of strains	215 (99·5)	1 (0·5)	187 (86·6)	29 (13·4)	100 (46·3)	59 (27·3)	57 (26·4)
No. of enterotoxigenic strains	86	0	79	7	46	23	17
Percentage of enterotoxigenic strains	40	0·0	42·2	24·1	46·0	39·0	29·8

( ) Percentage of total number of isolates.

* One hundred and seventy-nine breast milk samples (71·3%) obtained from 251 nursing mothers yielded 216 strains of staphylococci.

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and/or SEC (Table 2). There was no obvious association between the isolation of staphylococci, enterotoxigenic or not, and the incidence of diarrhoea in the babies (Table 1).

Two hundred and fifteen of the 216 isolates were coagulase-positive, and only one was coagulase-negative. The relationships between coagulase, thermonuclease, haemolysin and enterotoxin production are shown in Table 3. Generally, the frequency of enterotoxin production was highest among isolates that produced coagulase, thermonuclease and alpha-haemolysin, i.e. among the classic human *Staphylococcus aureus* strains.

DISCUSSION

The fact that 71·3% of breast milk samples from 251 healthy nursing mothers contained *S. aureus* is worrying, especially as 48% of the isolates produced enterotoxins. The incidence of enterotoxigenicity may even be higher, as we only looked for SEA, B and C. There are reports of SED and E-producing staphylococci from cases of mastitis elsewhere (Mochmann *et al.* 1979; Burbianka, 1971). It has been suggested that infants exposed to staphylococci in breast milk are at risk of staphylococcal infections and possibly infectio-toxicosis (Casman, 1965; Hallander & Korlof, 1967).

Burbianka & Dluzniewska (1971) reported that 89 (86·4%) of 103 strains of staphylococci from breast milk of mothers whose children were suffering from diarrhoea produced enterotoxin, and that as in the present study SEA predominated. These findings agree with published reports that SEA has been most frequently incriminated in staphylococcal food poisoning (Bergdoll, 1970, 1972). The overall frequency of 48% of samples yielding enterotoxigenic strains in the present study is higher than that reported by Mochmann *et al.* (1979). In that study 62 (31·2%) of 199 strains isolated from pus in cases of human mastitis produced enterotoxins. Also, all our mothers were apparently healthy, with no clinical mastitis, and were therefore breastfeeding their children.

There was no direct relationship between detection of enterotoxigenic staphylococci in breast milk and infantile diarrhoea. Sixteen mothers reported infantile diarrhoea. Of these, ten had staphylococci in their milk, but only six isolates were toxigenic. The number of children with diarrhoea was small, and this may have obscured any trend. Zak, Jeljaszewicz & Stochmal (1973) reported that 75·6% of isolates of staphylococci from diarrhoeal cases in Poland were enterotoxigenic, and that, together with the study by Burbianka & Dluzniewska (1971) cited earlier suggests that enterotoxigenic staphylococci may play some part in infantile diarrhoea. The number of cases of diarrhoea reported here is too small to resolve this problem and there is need for further work in this area.

Of more interest, we found a marked decrease in incidence of reported infantile diarrhoea with increasing age of the mother, from 10·5% of 57 mothers in the 16–20 age group to nil in 50 mothers over the age of 30, despite the fact that these two groups had an incidence of enterotoxigenic staphylococci in their breastmilk samples of 51·2% and 60·5% respectively. There are many factors which might explain the difference between these two groups of nursing mothers. It might be explained by greater experience in child caring and child bearing with age and

perhaps a greater appreciation of the importance of hygiene. There might also be a greater reluctance to admit illness in the child, or a readier acceptance of mild disturbance as normal. Nevertheless, this finding does suggest that the occurrence of enterotoxigenic staphylococci *per se* in breast milk does not determine the occurrence of infantile diarrhoea when the milk is consumed.

The correlation between coagulase and enterotoxin production found in this study is in agreement with published work by others (Bergdoll, 1972; Evans, Beuttner & Niven, 1950; Otenhajme & Mitra, 1974; Lachica, Weiss & Diebel, 1969). We also found that the incidence of enterotoxigenicity was higher (42·2 %) amongst thermonuclease-producing strains than the 24·1 % found among thermonuclease-negative strains. Lachica *et al.* (1969) had earlier reported a relationship between heat-stable nuclease and enterotoxin production.

Alpha haemolysin-producing strains were also more frequent producers of enterotoxins than either beta haemolysin-producing strains or those with a gamma haemolytic pattern. It was, however, surprising to observe that only 46·3 % of the 216 isolates produced alpha haemolysin while 27·3 % produced beta haemolysin. It has been documented that human biotypes produce predominantly alpha haemolysin while animal biotypes produce predominantly beta haemolysin (Elek & Levy, 1950; Hajek & Marsalek, 1969). The reason cannot easily be explained; previous work in this area (Adekeye, 1980), however, indicated that more than one biotype of *S. aureus* can be found on a particular host species. It is therefore possible that some of the beta haemolytic isolates were of animal rather than human origin.

In conclusion, data obtained from this study show that *S. aureus* can be isolated from a significant number of samples of milk from nursing mothers and that several of these isolates are enterotoxigenic. There is a need for a more detailed study of the role of these enterotoxigenic staphylococci in infantile diarrhoea.

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