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- Hospital staff

Ecology of Nasal Staphylococci

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ABSTRACT

DAVIS, NOUR A. (University of Lagos Medical School, Lagos, Nigeria), AND G. H. G. DAVIS. Ecology of nasal staphylococci. J. Bacteriol. 89:1163–1168. 1965.—The rate of nasal carriage of *Staphylococcus aureus* in Nigerian adults (46%) approximates that found in other countries. The rate in infants under 12 months was ca. 70%, which exceeds that found elsewhere, e.g., England. The incidence of penicillin resistance in nasal staphylococci (50 to 60%) is about the same as has been found in strains isolated from infections in outpatients in urban centers in this country. Mannitol-polymyxin agar was used for the selection and differentiation of coagulase-positive staphylococci and proved to be valuable in such studies. Our results clearly show that the degree of colonization by *S. aureus* significantly influences, or is influenced by, the rate of incidence of other bacteria in the vestibular flora, particularly in the case of diphtheroids and coagulase-negative cocci. The relationship between the degree of nasal microbial colonization and social and other factors is discussed.

Skin infections due to coagulase-positive staphylococci account for a very high proportion of the cases, especially children, presented as outpatients at hospitals and dispensaries in Nigeria. Staphylococcal pneumonia and other systemic involvements are also relatively common. Surveys carried out at Ibadan and Lagos (Collard, 1959; Davis, 1964) also indicate a high incidence of penicillin resistance (40 to 60%) in outpatients' strains of coagulase-positive staphylococci. Elek (1959) noted that the ecology of nasal carriage has been little studied; in view of these facts, the present work was initiated. In a previous paper, we evaluated some of the many selective media recommended for the primary detection of coagulase-positive staphylococci (Davis and Davis, J. Pathol. Bacteriol., *in press*). A modified version of Finegold and Sweeney's (1961) polymyxin agar incorporating mannitol and *p*H indicator was found most useful. The work reported here was largely dependent upon the use of this medium and was designed to give information on three aspects of the problem: (i) comparison of the nasal carrier rates in children, young adults, and hospital staff in Lagos; (ii) further evaluation of the usefulness of mannitol-polymyxin agar in such studies; (iii) obtaining a more accurate picture of the microorganisms associated with coagulase-positive staphylococci in the vestibular region of the nose.

MATERIALS AND METHODS

Swabs of the nasal vestibule were taken from 510 persons with cotton swabs moistened with saline. All of the subjects, with two exceptions in the hospital staff group, were African; 200 were nonhospitalized infants under 5 years. 200 were healthy adults between 18 and 30 years of age, and 110 were members of the hospital staff of a similar age group. All swabs were immediately inoculated onto blood-agar (5% human blood) and mannitol-polymyxin agar (MPA) of the following composition: nutrient broth (Oxoid CM1) made up as recommended by the manufacturers (Oxo Ltd., London, England), 1.5% (w/v) agar (Oxoid no. 3), 1% (w/v) mannitol, 500 units per ml of polymyxin B sulfate (Aerosporin, Burroughs Wellcome & Co., Inc., Tuckahoe, N.Y.), and 0.004% (w/v) bromocresol purple. The *p*H, unadjusted, was 7.4. The complete medium was autoclaved at 121 C for 15 min, and plates were prepared. Inoculation was achieved by firmly swabbing the agar surface, first on blood-agar and then on MPA. Growth was examined after 18 to 24 hr at 37 C. The type and amount of growth from each specimen on both media were recorded, and representative colonies were examined microscopically in Gram-stained preparations. All growth showing gram-positive cocci similar to staphylococci or micrococci was tested for coagulase activity by use of the slide method with undiluted human plasma. Those that gave negative results in the slide test were tested further by mixing one loopful of growth with 0.5 ml of undiluted plasma and were observed for 18 hr at 37 C (tube test). As many as possible of the coagulase-positive staphylococci detected by these means were tested for antibiotic sensitivity by use of Oxoid Multodisks (no. 11-14C), which incorporate the following agents: sulfafurazole, 100 μg; penicillin, 1.5 units; streptomycin, 10

TABLE 1. *Nasal carriage in infants, Lagos*

Age group	Male			Female			Sex not recorded			Grand total
	Degree of carriage		Total	Degree of carriage		Total	Degree of carriage		Total	
	Heavy	Light		Heavy	Light		Heavy	Light		
Under 1 year (151 tested)	26/53 (49%)*	10/53 (19%)	36/53 (68%)	27/59 (46%)	14/59 (23%)	41/59 (69%)	16/39 (41%)	12/39 (31%)	28/39 (72%)	105/151 (70%)
1 to 5 years (49 tested)	13/27 (48%)	3/27 (11%)	16/27 (59%)	8/22 (36%)	5/22 (23%)	13/22 (59%)	0	0	0	29/49 59%

* Numbers in parentheses are the percentage equivalent of each result.

TABLE 2. *Nasal carriage in young adults between 18 and 30 years of age*

Population group	Male			Female			Grand total
	Degree of carriage		Total	Degree of carriage		Total	
	Heavy	Light		Heavy	Light		
Hospital staff	8/25 (32%)*	1/25 (4%)	9/25 (36%)	37/85 (43%)	5/85 (5%)	42/85 (49%)	51/110 (46%)
Nonhospital adults	33/118 (28%)	9/118 (7%)	42/118 (35%)	47/82 (57%)	3/82 (3%)	50/82 (60%)	92/200 (46%)

* Numbers in parentheses are the percentage equivalents of each result.

μg; novobiocin, 5 μg; tetracycline, 10 μg; erythromycin, 10 μg; oleandomycin, 5 μg; chloramphenicol, 10 μg.

RESULTS

Incidence of nasal carriers. Tables 1 and 2 show an analysis of the incidence of nasal coagulase-positive staphylococci in the populations tested. When cultures were examined, the amount of growth of coagulase-positive cocci on MPA or blood-agar, or both, was recorded as confluent, moderate, or light. The moderate and light growths are combined under the heading of light growth (Tables 1 and 2). In practice, the isolation of any coagulase-positive cocci, whether light or heavy in growth, can be said to indicate carriage, because the cultural result can be influenced by uncontrollable discrepancies in the swabbing and plating technique and does not therefore necessarily reflect the degree of nasal colonization accurately.

According to Cunliffe (1949) and Rycroft and Williams (1960), nasal carriage of staphylococci in infants is usually highest in the first few weeks of life, and then declines to a low level around the age of 1 year before gradually rising with increasing age to the adult incidence rate. In English children, the maximal incidence ranges from 60 to 90% at 14 days after birth, the highest incidence occurring in babies born in a hospital. This falls to 10 to 20% at 1 year of age and rises to 30 to 50% at 5 years (Williams et al., 1960). None of the infants tested in our study was less than 1 month old. The incidence of carriers in the 1- to 6-month group (39 children tested) was 73%. In the 6- to 12-month group (112 children tested), it was 67%. These results are much higher than the comparable findings of Ryecroft and Williams (1960). The carrier rate in Lagos children of 1 to 5 years was approximately that found by the English workers.

Table 1 shows no difference in carrier rate between the sexes in young children. In Table 2, however, the results suggest that there may be a difference in carrier rates in young adults which is related to sex (*see* Millian et al., 1960). This is most noticeable in the nonhospital group. The overall percentage incidence of carriers in this age group (46%) agrees with findings in other countries (McDonald et al., 1960). Our comparison of hospital staff members, all of whom had been employed in clinical situations for at least 1 year, with nonhospital staff members (clerks, laundry workers, preclinical medical students, etc.) does

TABLE 3. *Antibiotic resistance of nasal coagulase-positive staphylococci*

Population†	No. of strains tested	Per cent resistance Ol							
		P*	S	T	C	E	G	Nv	Ol
Hospital-staff	45	80	57	26	11	7	100	2	7
Nonhospital adults	74	54	34	7	8	4	95	4	3
Children (0 to 5 years)	74	64	3	8	0	0	99	0	3

* Symbols: P, penicillin; S, streptomycin; T, tetracycline; C, chloramphenicol; E, erythromycin; G, sulphathiazole; Nv, novobiocin; Ol, oleandomycin.

† "Hospital staff" included only persons in close daily contact with patients, on wards or in operating theatres. "Nonhospital adults" included preclinical, medical, and nursing students, clerks, technicians, and laundry workers. All the children were seen at outpatient welfare clinics.

TABLE 4. *Comparison of cultural results for 510 nasal swabs on MPA and blood-agar*

Cultural result	MPA	Blood-agar
Pure growth of coagulase-positive staphylococci	248	100
Mixed growth of coagulase-positive staphylococci and other organisms	29	141
Growth of other organisms, inincluding coagulase-negative cocci	44	268
No growth	189	1
Total	510	510

not show that the higher rate of nasal carriage is in hospital personnel, which contradicts findings in other studies (Rountree and Barbour, 1951). In fact, in the female group, ca. 50% of the hospital staff were carriers versus 60% of nonhospital staff.

Antibiotic resistance in nasal coagulase-positive staphylococci. Table 3 shows the number of strains tested from each of three population groups and the percentage of resistance to individual antibacterial agents.

Efficiency of mannitol-polymyxin agar as a selective medium for coagulase-positive staphylococci. Previous work with mannitol-polymyxin agar (Davis and Davis, *in press*) showed that coagulase-positive staphylococci grew readily in 18 hr at 37 C as bright-yellow colonies, usually with yellow zones (indicator change) in the surrounding purple agar. Other organisms, especially other gram-positive cocci, were almost completely inhibited, and those that did grow were easily differentiated by their colonial morphology and lack of characteristic color reaction. In the present study, these findings were confirmed. In Table 4, we compared the cultural results on MPA and blood-agar for 510 nasal swabs. Coagulase-positive staphylococci were detected in 277 cases on MPA and in 241 on blood-agar. In most cases, the growth on MPA was pure, whereas, on blood-agar, the majority were mixed with other bacteria which confounded their recognition. The very marked reduction in growth of organisms other than coagulase-positive staphylococci on MPA compared with blood-agar is clearly shown in Table 4. In the 73 swabs that yielded other organisms on MPA, 22 gave gram-negative rods; 30, diphtheroids or streptococci; 5, coagulase-negative gram-positive cocci; 13, yeastlike fungi; and 3, gram-negative cocci. The gram-negative rods were mainly *Proteus* sp. and were easily recognizable, although they did not swarm on MPA. Two of these gram-negative strains produced brown-yellow mucoid colonies quite unlike coagulase-positive staphylococci. None of the other organisms produced yellow growth, and, in the case of the diphtheroids, streptococci, coagulase-negative cocci, and neisseriae, the colonies were of the pinpoint colorless type and were frequently difficult to see with the naked eye. Many of the yeastlike fungi were strains of *Candida tropicalis*, with white granular colonies.

On blood-agar, over 50% of the swabs yielded detectable coagulase-negative cocci; 190, diphtheroids; 100, gram-negative rods; 9, gram-positive bacilli; 5, yeast; 8, streptococci; and 45, neisseria.

These results show the selective value of MPA for coagulase-positive staphylococci. Of the 277 coagulase-positive cocci detected on MPA, 258 (92%) showed characteristic, good colonies, bright yellow in color and usually with yellow zones in the surrounding agar. The remaining 19 strains (8%) grew as good colonies, but these were white or only faintly yellow after 18 hr at 37 C. All except one of these were from plates with fewer than 20 colonies, i.e., scanty growth. Prolonged incubation (3 days) of these cultures produced a stronger yellow color in most cases. In 228 cases, coagulase-positive cocci were detected on both MPA and blood-agar. In 49 cases, coagulase-positive cocci were detected on MPA but not blood-agar, and, in 12 cases, on blood-agar but not on MPA. This last result may have been due to the fact that the blood-agar was always inoculated first, and it is also possible that undetected mixed growth from blood-agar may have caused false coagulase-positive reactions in a few cases.

TABLE 5. *Relationship between the degree of growth of coagulase-positive staphylococci and the presence of other microorganisms in 510 cultures of nasal swabs*

Microorganism	Confluent or heavy* (165 swabs)				Moderate (49 swabs)				Light (64 swabs)				No growth (232 swabs)			
	C† (62)	H (33)	NH (70)	Total (165)	C (26)	H (12)	NH (11)	Total (49)	C (45)	H (7)	NH (12)	Total (64)	C (67)	H (58)	NH (107)	Total (232)
Pure coagulase-positive staphylococci	50‡	49	57	52	11	16	27	16	0	14	8	3	0	0	0	0
Coagulase-negative cocci	9	42	8	16	58	75	36	57	69	86	67	70	78	86	86	84
Diphtheroids	19	15	10	16	46	16	27	35	55	14	50	50	76	30	50	48
Neisseria-like cocci	9	0	0	4	38	0	0	20	31	0	0	22	40	0	0	12
Gram-negative rods	25	51	24	22	11	8	33	10	22	28	58	30	27	12	42	30
Streptococci	3	0	0	1	12	0	0	6	9	14	0	8	14	9	2	7
Yeasts	5	0	0	2	8	0	0	4	13	0	0	9	10	0	0	3
Gram-positive rods	1	6	1	2	8	0	18	8	11	0	0	8	10	5	4	6

* Nasal swabs were divided into four groups according to the degree of growth of coagulase-positive staphylococci.

† Symbols: C, children, 0 to 5 years; H, hospital staff; NH, nonhospital staff.

‡ Results are the percentage of nasal swabs which yielded growth of the various microorganisms.

Microorganisms associated with coagulase-positive staphylococci in the nasal vestibule. In this study, no attempt was made to classify the microorganisms of the vestibular flora beyond a differentiation into broad morphological groups based on microscopy and the detection of coagulase activity in gram-positive cocci. The groups were as follows: coagulase-positive staphylococci (*Staphylococcus aureus*, *Bergey's Manual*); coagulase-negative cocci, which includes staphylococci, micrococci, and possibly other types; diphtheroids, the majority of which were assumed to be members of the genus *Corynebacterium* (Moore and Davis, 1963), although a few resembled *Nocardia* of the form unlike *Streptomyces* (Davis and Freer, 1960); gram-negative cocci of the *Neisseria* type, some of which may be members of other genera, e.g., *Moraxella*, *Mima;* gram-negative rods, including members of the Enterobacteriaceae (*Klebsiella*, *Proteus*, and coliforms), pseudomonads, and haemophili; streptococci, including pneumococci; yeastlike fungi, most of which were probably *Candida* sp.; gram-positive rods, excluding obvious sporing bacilli on the grounds that they are almost certain to be casual contaminants and not residents of the nose. The methods used for culturing swabs precluded the growth of anaerobes, which are numerous in the nasal passages and probably also in the vestibule (Watson et al., 1962).

Table 5 shows an analysis of our results in terms of the percentage of swabs which yielded growth of the organisms listed above and their distribution in relation to the degree of growth of coagulase-positive staphylococci, and also the population group from which they emanated. The following main points emerged from this. Assuming that the cultural result reflects more or less accurately the microbial ecological state of the area swabbed, the presence of large numbers of coagulase-positive staphylococci results in simplification of the resident flora. Of the cultures showing confluent or heavy growth of coagulase-positive staphylococci, 50% were apparently pure, but the percentage of pure cultures fell drastically and progressively when the growth of coagulase-positive staphylococci was only moderate or light. This can also be deduced by observing that the percentage incidence of other organisms rises progressively if one reads the columns headed "Total" from left to right across the table. As the incidence of coagulase-positive cocci falls, the incidence of almost all the other organisms rises. Similar trends can be seen in some of the specific population group columns, i.e. the incidence of coagulase-negative cocci in all three population groups, and of diphtheroids in children and nonhospital staff.

Coagulase-negative cocci, diphtheroids, and gram-negative rods were the most commonly encountered organisms other than *S. aureus*. The distribution of gram-negative rods did not appear to be related to either the incidence of *S. aureus* or the population groups. Neisseriae were only found in the children, and the incidence showed only dubious evidence of an inverse relationship with coagulase-positive staphylococci. The percentage incidence of streptococci, yeasts, and gram-positive bacilli was probably too low for any significance to be attached to it in a study of this

nature. Of the three populations examined, the infant group exhibited by far the most complex flora and the hospital staff the simplest; 46% of the children yielded three or more different microorganisms in culture, whereas only 8% of the hospital adults did so. It was also noted that in the nonhospital staff group more complex floras were encountered in manual workers (e.g., laundry men) than in clerical workers.

DISCUSSION

Apart from an apparently high rate of nasal carriage in infants in the 6- to 12-month age group and a possible sexual difference in adult carrier rates, our results relating to the nasal carriage of *S. aureus* in Nigeria conform with those of workers in other countries. Findley and Abrahams (1946) reported a lower rate of nasal and skin carriage in Africans compared with Europeans in Ghana.

Antibiotic resistance, particularly to penicillin, in strains of *S. aureus* isolated from outpatients in Nigeria is known to be high (Davis, 1964), and this was also true of the strains isolated from healthy persons in this study.

MPA proved to be a very useful and reliable selective and differential medium for coagulase-positive staphylococci found in the nose, and it is very easy to prepare and store. Workers in the field of food microbiology (Jay, 1963; Baird-Parker, 1962) have reported adversely upon the value of polymyxin agar in that field, but our experience supports that of Finegold and Sweeney (1961) and Polster (*personal communication*), who reported its value in medical bacteriology.

It has been known for many years that competition between microorganisms plays an important role in determining the qualitative and quantitative composition of the microbial floras of man. Rosebury (1962) has reviewed this field comprehensively, and Elek (1959) should be consulted for a more detailed account of the interactive phenomena associated with *S. aureus*. The results shown in Table 5 confirm those of O'Grady and Willstandt (1963), who reported that other bacteria were less commonly found in the noses of coagulase-positive *Staphylococcus* carriers. Our own results suggest that this is a progressive phenomenon correlating with the quantitative incidence of coagulase-positive staphylococci. The antagonistic activity of *S. aureus* toward other bacteria, notably corynebacteria and other gram-positive bacteria, has been investigated by various workers. Barrow (1963*a*, *b*) reported the isolation of an antibiotic agent related to this particular activity.

Nasal hygiene is probably also an important factor in determining the flora found in any particular subject. As already noted, complex nasal floras are most common in infants, relatively common in manual workers, and least common in the higher social and educational groups. Apart from the actual ability or desire to practice nasal hygiene, there is also the factor of exposure to contamination. It is almost certain that those two factors operate in conjunction to yield the observed results. As Rosebury (1962) remarks, the anterior nares probably collect microorganisms in much the same way as do the finger nails. The parallel is a useful one when viewing the nasal flora in terms of age and social level (*see* Miller and Jones, 1964). It is noteworthy that the most common single cause of death in infants in Nigeria is respiratory infection, and the organisms most frequently isolated postmortem are *Klebsiella* spp., *Proteus* spp., coliforms, pseudomonads, *Haemophilus* spp., streptococci, and staphylococci (E. O. Odunjo, University of Lagos Medical School, *personal communication*). All these organisms occur in infants' noses, and the relationship between the nasal flora and respiratory disease warrants further study.

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