Data Regarding the Enteropathogenicity of Staphylococci in Infants

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Dyspeptic disorders have always been an important problem in paediatrics, — especially as the causative agent cannot be always identified. In cases of unclear aetiology the responsibility of staphylococci has been suggested, these organisms being capable of producing disease ranging in severity from mild dyspepsia to the so-called choleriform syndrome [11] and chronic enteritis.

In previous studies [1] we could isolate staphylococci from the faeces of 30 per cent of the infants admitted to our Department or treated there as out-patients. This incidence is comparable to data in the literature [18].

In the present investigations we have undertaken to study the role played by the staphylococci isolated from the faeces of sick infants. The patient material of our Department has therefore been subjected to a systematic study. The biological properties of the staphylococci isolated from faecal samples have been determined and analysed for eventual correlation with the clinical symptoms. The questions to which we have sought the answers were as follows.

(i) What are the biological properties of the staphylococci isolated from the faeces?

(ii) Do staphylococci play a role in the pathogenesis of infantile enterocolitis of unknown aetiology?

(iii) Is the use of broad-spectrum antibiotics followed by a settlement of staphylococci in the intestinal tract?

(iv) Is there a correlation between the stay at the Department and the presence of staphylococci in the faeces?

(v) Is there a difference concerning the incidence of positive bacteriological findings between the "enterocolitis" and the "respiratory infection" wards?

Material and Methods

Material. In the period June 5 to August 20, 1959, 126 infants were studied. Forty-nine were admitted with enteral complaints and 77 suffered from respiratory or other diseases. The latter group served as the control. Changes in the intestinal bacterial flora were recorded from admission until discharge. The infants ranged in age from 2 weeks to 1 year.

Bacteriology. Faecal samples were taken every other day and inoculated into 8 per cent NaCl agar, Endo and DC culture media, to demonstrate *Staphylococcus, E. coli* and *Shigella.* The number of staphylococcus colonies in the medium was indicated by plus signs, + meaning 1 to 10 colonies, ++ 10 to 50, and +++ 50 or more.

The strains exhibiting the characteristic microscopic pattern and colony morphology, reducing nitrate and not utilising ammonium phosphate as a source of nitrogen, were accepted as being *Staphylococcus aureus* [4]. The pathogenicity of the strains was concluded upon by the demonstration of the enzymes coagulase, hyaluronidase and phosphatase.

Sensitivity to antibiotics was determined by the paper disc method, against penicillin, streptomycin, chloramphenicol, aureomycin, terramycin, neomycin, erythromycin and sulphonamides.

Serology. Typing with factor sera a, b, c, e, f, h, i, k was carried out according to OEDING [16]. Live and autoclaved strains were agglutinated on slides. The type of the strain was determined on the basis of the part antigens demonstrated and the typed strains were grouped. Earlier, OEDING [16] and GRÜN [9] had considered the antigens a, b, c, e, to be group antigens and f, h, i, k to be type antigens. Recently, OEDING and WILLIAMS [17] have recommended a new classification on the basis of a comparison of phage and serological types and distinguished four serological groups. The characteristic antigens of the four groups are as follows.

Group 1. is characterized by the presence of antigen "e".

Group 2. is characterized by the absence of antigens "e", "i" and "k".

Group 3. is characterized by the presence of antigen "i".

Group 4. is characterized by the presence of antigen "k".

Within the single groups, beside the group antigens mentioned, any of the part antigens (a, b, c, f, h) may occur as type antigen.

The method employed has been described in detail [2, 22].

The *Escherichia coli* strains grown on Endo medium were identified in OB sera.

RESULTS

Bacteriology and Serology

A total of 540 faecal samples and from repeated inoculations 249 strains of staphylococcus were isolated. The results of the *pathogenicity* tests are presented in Table I. The coagulase, hyaluronidase and phosphatase tests were equally positive in the case of 230 strains. Of the remaining 19 strains 8 strains were coagulase positive, hyaluronidase negative, and 4 strains coagulase positive, phosphatase negative. Of the 7 coagulase negative strains 6 gave positive hyaluronidase and phosphatase tests and one was found to produce hyaluronidase only. According to our earlier experience [2], the presence of coagulase is the most reliable determinant of pathogeni-

TABLE I

The results of pathogenicity tests for 249 strains of Staphylococcus aureus

Phosphatase	Hyaluronidase	Coagulase	Number of strains
+	+	+	230
+	−	+	8
−	+	+	4
+	+	−	6
−	+	−	1
		Total	249

city. On this basis, 242 strains of the 249 were potential pathogens.

The results for the sensitivity to antibiotics are shown in *Fig. 1.* Only 8.4 per cent of the strains were sensitive to penicillin, 14.8 per cent were sensitive to streptomycin and 17 per cent to chloromycetin. Aureomycin, and terramycin were active on 19 per cent of the strains. Every strain was sensitive to neomycin and 43.8 per cent to erythromycin.

From the data of the sensitivity tests was determined the *antibiogram* of the strains. They were classified into two large groups (Table II),

1. "polysensitive" strains, and
2. "polyresistant" strains.

Polysensitive were 15.6 per cent of the strains; they were sensitive to tetracyclins, neomycin and erythromycin. The few penicillin and streptomycin sensitive strains also belonged to this group. Polyresistant strains occurred in 84.4 per cent. On the basis of the antibiograms, these were classified into 3 subgroups, *viz.*

a) sensitive to neomycin only, 137, 55 per cent,

b) sensitive to neomycin and erythromycin, 70, 28.2 per cent,

c) sensitive to neomycin and chloromycetin, 3, 1.2 per cent,

84.4 per cent.

The serological results are presented in Table III. Eleven strains (4.4 per cent), each "abce"-type, belonged to serological group 1. Group 2. strains numbered 145 (58.2 per cent), including 8 different types, notably "(a)b",

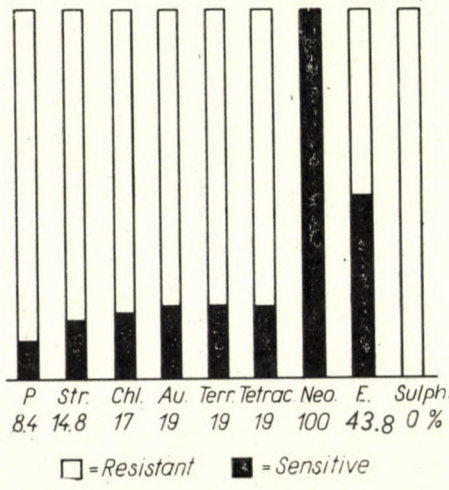

FIG. 1. Antibiotic sensitivity of strains, per cent

TABLE II

Percentage distribution of 249 strains of *Staphylococcus aureus*, according to the antibiogram

Antibiotic	Polysensitive 15.6 %				Polyresistant		1.2%
					55%	18.2%	
Penicillin	●	○	○	○	○	○	○
Streptomycin	●	●	○	○	○	○	○
Chloramphenicol	●	●	●	○	○	○	●
Aureomycin	●	●	●	●	○	○	○
Terramycin	●	●	●	●	○	○	○
Neomycin	●	●	●	●	●	●	●
Erythromycin	●	●	●	●	○	●	○
Sulphonamides	○	○	○	○	○	○	○
Total	15.6%				84.4%		

● sensitive ○ resistant

TABLE III

Serological distribution of *Staphylococcus aureus* strains

Serological group							
1.		2.		3.		4.	
Type	Number of strains	Type	Number of strains	Type	Number of strains	Type	Number of strains
abce	11	abc	37	—	—	abce(f)hk	5
		bc	55			abck	5
		(a)b	6			abk	2
		c	37			abcek	1
		bcf	1			bck	1
		bf	2			bfk	2
		f	9			abfk	2
		a	1				
Total	11	Total	148	Total	0	Total	18

"bc", "abc", "c", "bcf", "bf", "f", and "a". None of the strains tested belonged to group 3. Group 4. was represented by 18 strains including the 7 types "abce(f)", "hk", "bck","abk","bfk","abck","abcek", "abfk". As already reported [22], we were the first to observe the type significance of the antigen "f". Its frequent occurrence was observed also in the present studies.

The types occurring most fre-

quently were "bc" (55), "abc" (37), and "c" (37). Of the 249 strains 136 (54.6 per cent) belonged to one of these types.

Inagglutinable were 30.2 per cent of the strains.

A comparative analysis of the *serological types* and the *antibiogram* yielded the following remarkable result.

To the *polysensitive* strains belonged:

the group 1. strains

of the group 2. strains, those of type "bcf", "bf", "a" and "f";

the group 4. strains, except for 3 strains; and

3 of the inagglutinable strains.

The *polyresistant* strains were

of group 2. the "(a)b", "abc", "bc" and "c" type strains;

of group 4. 3 strains ("abck"); and 72 of the inagglutinable strains.

These data are illustrated in Table IV. The serological types encountered showed a consequent behaviour also on the basis of the antibiogram. There were only 2 exceptions: 3 strains "abck" of serogroup 4, and 3 inagglutinable strains.

E. coli strains occurred in all 540 faecal samples; from 32 samples strain 0111: B4 was isolated. Bacteria of the Shigella group were not encountered.

TABLE IV

Correlation between serological types and antibiogram

Antibiogram	Serological groups	Serotype	1.	Inaggl.	2.								3.	4.							
			abce	—	abc	bc	c	(a)b	a	bef	bf	f	—	abck	abcek	abfk	bfk	bck	abcefhk	abk
Polyresistant				72	37	55	37	6					—	3						
Polysensitive			11	3	—	—	—	—	1	1	2	6	—	2	1	2	2	1	5	2

CLINICAL OBSERVATIONS

Of the 49 cases of enterocolitis 14 were due to *E. coli 0111: B4;* 35 were of unknown aetiology. Dysentery did

not occur during the observation period.

Of the 14 cases due to *E. coli 0111: B4*, staphylococci were isolated from the faeces of 4 infants immediately after admission, while from 9 infants later during their stay at the Department; 1 patient remained negative throughout.

Among the 35 cases of enterocolitis of unknown aetiology 9 showed the presence of staphylococci in the first faecal sample (+, ++); 11 turned positive while at the Department; 15 remained negative throughout.

Thus, staphylococci were demonstrated in the faeces at the first examination in 13 of the patients with enterocolitis. This figure subsequently increased to 33.

The infants suffering from diseases other than enterocolitis (respiratory infection, pneumonia, otitis, etc.) totalled 77. Staphylococci were isolated from the faeces of 16 patients at the first examination. This figure later increased to 35, while 42 infants remained negative throughout.

The difference in the incidence of positive cases between the enteritis and respiratory groups was not significant ($\chi^2 = 0.34$, $0.20 < P < 0.50$). The same applies to the number of infants that had turned positive during their stay at the Department ($\chi^2 = 1.88$, $0.20 > P > 0.10$).

These data indicate that staphylococci occurred in the intestinal tract independently from the clinical disease.

The presence of staphylococci in the faeces did not influence the clinical picture. This was true in all of the cases observed, in the enterocolitis and the control group alike. In view of this it is only natural that no correlation was found between the clinical picture and the number and serological type of the bacteria grown, either.

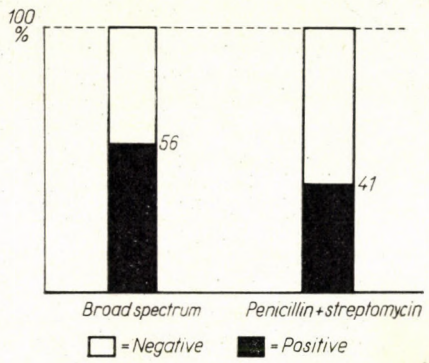

FIG. 2. Percentage correlation between antibiotic therapy and stool positivity

The above data make it clear why we could not ascribe significance to the presence or absence of staphylococci in the faeces as regards the development or lack of dyspepsia. However, the high percentage of staphylococcus-positive samples induced us to carry out further investigations.

First, the effect of antibiotic treatment on the intestinal flora was investigated. Of the 126 infants 84 (67 per cent) were treated with broad-spectrum antibiotics while 39 (31 per cent) were treated with penicillin and streptomycin or erythromycin. The incidence of staphylococcus-positives was 56 per cent in the former group

and 41 per cent in the latter. As shown also in *Fig. 2*, there was no significant difference between these two groups. The percentage of staphylococcus-positives was almost identical in the two groups, independently of the antibiotic administered.

The duration of antibiotic treatment and the doses employed had no influence on the incidence of staphylococcus-positive stools, though the antibiotics given before admission were also taken into consideration. Three infants had not been given drugs and in spite of this 2 of them had staphylococcus-positive stools.

Thus, the frequent occurrence of staphylococci in the faeces could not be explained by a previous antibiotic treatment, and such treatment was not regularly followed by the appearance of staphylococci in the faeces. In the positive cases it seemed to be of no importance whether the infants had been treated with or without broad-spectrum antibiotics.

Next, the eventual correlation between the positive findings and the duration of stay at the Department was studied. Staphylococci were demonstrated in the faeces by the first test already in 29 (23 per cent) of the 126 infants, independently of the previous antibiotic treatment. However, the number of positive samples continued to increase with the length of stay at the hospital. When discharged, 51 per cent of the infants were positive. Analysing the details it was found that most of the infants had turned positive during the first week at the Department. This is illustrated graphically in *Fig. 3*, which in addition shows the number of cases infected at the Department during the 12 days of observation. Frequency increased steeply during the first days, then the rise was more gradual.

Serological typing revealed that from the stool samples turned positive after admission exclusively types "(a)b", "abc", "bc", and "c" of serogroup 2., and except for 2 cases the inagglutinable strains were isolated. As already mentioned, these types had a characteristic antibiogram, they were sensitive to neomycin and erythromycin only. In contrast with this, the strains isolated immediately after admission belonged to serogroups 1. and 4., or were type "bcf", "bf" and "a" of serogroup 2. These strains were sensitive to most antibiotics.

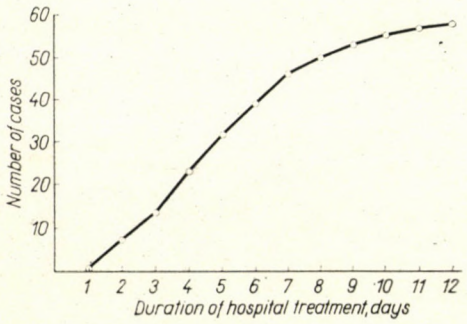

FIG. 3. Infection with nosocomial strains during stay in hospital

In the 29 cases which had been positive at admission the following observations were made. In 7 cases the infant retained the strain he had

brought with him and in 22 infants antibiotic treatment caused a disappearance of the staphylococcus. Of these 22 infants in 16 the cultures were negative for 2 to 4 days, then group 2. strains or the inagglutinable strain characteristic of hospital infection appeared, usually with increasing intensity (+, ++, +++) and with the characteristic antibiogram. The remaining 6 cases remained negative after antibiotic treatment.

In the case shown in Table V is illustrated the disappearance of the strain the infant had brought with him and the appearance, after a few days of latency, of the nosocomial strain. The occurrence was characteristic of both the control and the enterocolitis groups. When discharged, of the 67 infants of the control group 30 (44 per cent) had staphylococcus positive faeces and 37 patients (56 per cent), were negative. Of the 59 infants in the enterocolitis group 35 (59 per cent) were positive and 24 (41 per cent) negative at discharge.

The difference between the data was not significant. This means that the two wards did not differ in staphylococcal incidence. In both wards staphylococci had been isolated from the faeces of nearly 50 per cent of the infants and as related to the number of the positives at admission the percentage of staphylococcus excretors increased in the same measure, by 34 per cent in the enteritis wards and 29 in the control wards. This finding supports the view that staphylococci were spread by the same mechanism in both groups.

Discussion

Our investigations supplied the following answers to the questions raised in the introduction.

ad (i). The strains isolated from the faeces were essentially identical in biological properties with those originating from pyogenic processes. The staphylococcus strains isolated were potential pathogens.

The majority of the strains isolated were resistant to antibiotics. A similar measure of resistance has been reported by Djurisic and Drndarski [8], who found 69 per cent of their staphylococcus strains resistant to penicillin, 71 per cent to streptomycin and 46.5 per cent to terramycin. Of the antibiotics tested by us only neomycin was effective against every strain isolated. The number of strains resistant to erythromycin was remarkably high (50.7 per cent).

The high incidence (30.2 per cent) of inagglutinable strains was remarkable. Among the strains isolated by Grün [9] from faeces, 19 per cent were inagglutinable, and among those we had isolated earlier, 26.4 per cent [1]. This was unexpected, as the strains from other specimens, for instance pyogenic infections, are usually inagglutinable in only 1 to 3 per cent. We are at present unable to explain this high incidence of inagglutinable strains. We may have dealt with a new antigen or the phenomenon may have been a consequence of the marked antibiotic resistance (phage typing by Váczi *et al.* [23]).

The epidemiology of staphylococci

TABLE V

Appearance of nosocomial staphylococcus strain in the faeces after antibiotic treatment

Date of isolation June	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Antibiogram P	○			○		○		○			○		○							○		⊙			⊙
Str	●			●		●		●			●		●							○		○			○
Chl	●			●		●		●			●		●							○		○			⊙
Au	●			●		●		●			●		●							○		○			○
Terra	●			●		●		●			●		●							○		⊙			○
Neo	●			●		●		●			●		●							●		●			●
E	●			●		●		●			●		●							●		●			●
Sul	○			○		○		○			○		○							○		○			○
Number of colonies	+			+		+++		+++			+++		+++		—			—		++		+++			+++
Serogroup	1			1		1		1			1		1		—			—		2		2			2
Serotype	abce			abce		abce		abce			abce		abce		—			—		abc		abc			abc

● sensitive ○ resistant |——| terramycin treatment

may be studied by serological methods, phage typing and antibiogram studies. Phage typing will produce results in 30 to 50 per cent of the cases only, because of the large number of strains that cannot be typed. As in the present instance serological testing presented some difficulties, we felt it imperative to employ simultaneously at least two of the three methods. The results obtained by the two methods were in excellent agreement.

The type classification of OEDING and WILLIAMS [17], as mentioned above, is based on a comparison of the results of phage- and sero-typing. The evidence now obtained has proved the practical value of this classification

ad (ii). In our investigations particular attention has been focussed upon the cases of enteritis of unclear aetiology. However, staphylococcus enteritis did not occur among the 36 similar cases. Thus, our material did not support the aetiological role of staphylococci in enteritis. According to KIENITZ [12] the staphylococcus would induce enteritis exclusively when it is demonstrable in pure culture on non-selective media, in patients presenting characteristic clinical symptoms. We are unable to accept this view, because it may occur that antibiotic treatment destroys the sensitive *E. coli* (and the other members of Enterobacteriaceae) and at the same time the acquired, resistant staphylococci appear in pure culture in the commonly used media. Acquiration of resistant hospital strains offers an excellent possibility for this. The result will be that the preponderance of staphylococci may give one the impression that the enteritis is due to them. The picture may become even more complicated by the development of chloramphenicol enteritis. We do not wish to deny that chloramphenicol enteritis may have a bacteriological background, but it should be borne in mind that the Enterobacteriaceae and staphylococci are merely a minute fraction of the intestinal flora.

It has been claimed [13] that staphylococcal enteritis cannot be diagnosed unless the presence of enterotoxin is demonstrated. TOLENTINO [21] found, however, merely 24 per cent of the staphylococci isolated from cases of staphylococcal enteritis to be enterotoxin-producers. It therefore appears that the strains causing enteritis need not produce enterotoxin. On the other hand, demonstration of the toxin is difficult because of the special conditions required for its production and the variable tolerance of the test animals.

Pure cultures of staphylococci never occurred in the faeces of our patients. As none of our cases had been suspected to be one of staphylococcal enteritis, tests to demonstrate this toxin were deemed unnecessary.

It is therefore suggested that the staphylococci producing enterotoxin and growing in pure culture should be made responsible for the enteritis exclusively when certain other factors, too, have been taken into con-

sideration (*e. g.* by a qualitative study of the intestinal flora), resp. ruled out (*e. g.* chloramphenicol or virus enteritis).

ad (iii). Although in our cases the aetiological role of the intestinal staphylococci could not be proved, the number of positive results was remarkably high. Still, in view of the data published by METZGER *et al.* [15], as well as by LOK and BACHER [14], that broad-spectrum antibiotics may cause an increase of staphylococci in the intestinal tract, we felt justified to surmise that the high incidence of these positive results might have been due to the antibiotic treatment. A comparison of the results for the infants treated with penicillin with those for the infants treated with broad-spectrum antibiotics showed, however, no significant difference between the two groups, as staphylococci were isolated from about 50 per cent in both. This has ruled out the effect of antibiotic therapy.

ad (iv). Next, we studied the correlation between the duration of stay at the Department and the presence of staphylococci in the faeces, with due regard to the biological properties of the strains. It was namely found that the strains of serogroup 2. and types "(a)b", "abc", "bc", and "c", as well as inagglutinable strains occurred in a very high percentage. These strains appeared on the second to ninth days of hospital treatment. As the serotypes and antibiograms were characteristic and their appearance was systematic, the strains were considered nosocomial.

There is a certain parallelism between the changes in the faecal and the naso-pharyngeal staphylococcus flora. It is well-known that during hospital treatment nosocomial strains can usually be isolated from the nose and throat of the patient. Other data indicate that the staphylococci in the faeces originate from the nasopharynx [5, 6], and this explains why so many hospitalized patients excrete staphylococci with their faeces. The nosocomial strains reach the nasopharynx presumably through the air, dust, utensils [19] and then spread to the upper parts of the gastrointestinal tract. The nosocomial strains occur in smaller number in the intestinal flora than in the nasopharynx, where they are present in about 80 to 90 per cent of the patients. This is easy to understand if it is realized how many barriers (gastric acidity, etc.) have to be passed before reaching the intestines. There are also considerable individual variations in this respect [14].

ad (v). Our studies were carried out in two wards. The control infants with respiratory infection developed no enteritis, in spite of their having contracted the nosocomial staphylococcus in about the same percentage as the infants admitted with enteritis.

The agreement between the positive findings is indicative of an identical mechanism. The situation is probably similar in other departments and wards and it seems that infection by the nosocomial staphylococcus strains *in se* does not induce enteritis or any other clinical symptom.

Summary

(i) The enteropathogenic role of staphylococci has been studied in 126 infants.

(ii) The incidence of staphylococci in the faeces of patients with enteritis of known and unclear aetiology was practically the same.

a) At admission the incidence of staphylococci in the faeces was not lower in the patients with respiratory infection than in those with enteritis.

b) Staphylococcus infection acquired in the hospital induced neither enteritis nor other clinical symptoms. Under the conditions of the present study the staphylococci had no enteropathogenicity.

(iii) The incidence of staphylococcus positivity increased with the duration of stay at Hospital. Serological typing and antibiograms showed the increase to be due to infection with nosocomial strains.

(iv) During the test period the nosocomial strains were resistant to the antibiotics used.

(v) The acquired strains are presumed to play a role in the increased incidence of resistant staphylococci.

(vi) The problems of diagnosing staphylococcal enteritis have been discussed.

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