MISLEADING SPUTUM CARTONS

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(RECEIVED FOR PUBLICATION JANUARY 19, 1960)

Waxed paper sputum cartons obtained from the manufacturers contained very few organisms. Similar cartons which had been stored in hospital wards were often contaminated with hospital dust. This contamination was sometimes sufficiently marked to be misleading. Coliform organisms are particularly liable to cause confusion.

Waxed cartons are useful for collecting sputum, but they must be properly stored.

In many hospitals waxed paper cartons are used for collecting specimens of sputum. Recently we observed substantial deposits of London soot in some unused cartons stored in a ward, and it seemed possible that significant numbers of airborne microbes might also be gaining access to the cartons. A number of unused cartons were therefore sampled to find out whether they contained any organisms, and, if so, whether these were likely to be misleading.

Methods

Source of Cartons.—The cartons were supplied by the manufacturers in batches of 1,000 packed in a cardboard case. The cartons themselves were not individually wrapped, but the lids were wrapped in paper in batches of 20. In the wards the cartons were kept in cupboards, sometimes enclosed in the paper bags in which they were issued by the stores though often exposed to the air in trays or boxes. The lids were usually kept in their original wrappings.

Over a period of three months 100 cartons were collected from the wards and 100 cartons from the stores. Those from the stores included 50 cartons taken at random and 50 obtained from a new case of cartons which we opened ourselves. Lids were placed on the cartons at the time of collection.

Sampling of Cartons.—Digest broth, 2 ml., was added to each carton, and with the lid held on firmly the contents were swirled vigorously 10 times. A sample of the washings (usually 0.5 ml.) was then spread on blood agar plates which had been freshly poured and dried. The plates were inspected after aerobic incubation for 24 hours at 37° C. and re-inspected after a further 24 hours at room temperature. The different types of colony on each plate were noted and representative colonies were identified. The total number of colonies on each plate and the different types of organism which made

up this total were counted. The number of organisms in each carton was calculated.

Artificial Contamination of Cartons.—A patient with exfoliative dermatitis, known to disseminate vast numbers of *Staphylococcus aureus*, was used as the source of this organism. Large numbers of cartons from a new batch known to contain very few organisms were exposed in his room while the bed was made and the room dusted. From colony counts on blood agar plates exposed at the same time the probable number of organisms falling into each carton was calculated. To prepare cartons containing *Bacterium coli* suitable dust was generated by shaking pieces of cotton cloth which had been impregnated with a broth culture and then dried for 24 hours at 37° C.

Results

Table I summarizes the average counts obtained by sampling 100 cartons from the wards and 100 cartons from the stores. It will be seen that very few organisms were recovered from 50 cartons from a new batch obtained straight from the manufacturers. Higher counts were obtained from 50 cartons collected at random from the stores, suggesting that some contamination occurred in this building. However, these counts were very much smaller than counts obtained from cartons from the wards.

TABLE I
AVERAGE NUMBER OF ORGANISMS ISOLATED FROM
UNUSED SPUTUM CARTON

Source of Cartons	No. Sampled	Average No. of Organisms per Carton
Medical and surgical wards . .	100	419
Central stores		
(a) At random	50	44
(b) New batch	50	3

The counts of total organisms from individual cartons varied greatly and are analysed further in Table II. It will be noted that 70% of the cartons obtained from the stores were sterile compared with only 4% of those obtained from the wards. At the other end of the scale no carton from the stores contained more than 1,000 organisms, but six cartons from the wards gave counts higher than this.

TABLE II
DISTRIBUTION OF COUNTS OF TOTAL ORGANISMS

	Total No. of Organisms per Carton					
	Sterile	1-10	11-100	101-1,000	1,001-10,000	>10,000
No. of cartons in each category from:						
Wards ..	4	11	44	35	5	1
Stores ..	70	15	9	6	0	0

The flora of contaminated cartons usually consisted of several different types of organism. To indicate the types most frequently encountered a record was made of the number of cartons which were shown by differential counts to contain more than 100 organisms of the same type. Of the 200 cartons sampled, two contained more than 100 of each of three different types of organisms, eight contained more than 100 of two different types, and 26 contained more than 100 of only a single type. The predominant organisms isolated from these 36 cartons are shown in Table III.

TABLE III
NUMBER OF CARTONS CONTAINING MORE THAN 100 ORGANISMS OF THE SAME TYPE

Type of Organism	Cartons from Wards			Cartons from Stores		
	101-500	501-1,000	>1,000	101-500	501-1,000	>1,000
	Organisms/ Carton			Organisms/ Carton		
<i>Staph. aureus</i> ..	4	0	1	0	0	0
Other staphylococci ..	15	1	2	3	0	0
<i>Bact. coli</i> ..	1	0	1	0	0	0
Other coliforms ..	1	1	1	0	0	0
<i>Streptococcus</i> spp. ..	3	1	0	1	0	0
<i>Neisseria</i> spp. ..	1	0	0	2	1	0
<i>Bacillus</i> spp. ..	2	0	1	1	0	0
Moulds ..	3	1	0	0	0	0

The organisms most commonly encountered were staphylococci. Most of these were coagulase-negative strains variously considered as belonging to the genera *Micrococcus*, *Sarcina*, and *Staphylococcus*, but five cartons from the wards contained more than 100 coagulase-positive *Staph. aureus*, one particular carton containing no less

than 2,400 *Staph. aureus* out of a total flora of 5,400 organisms.

Two cartons contained more than 100 typical *Bact. coli*. One of these cartons was the most heavily contaminated in the whole series. Out of a total flora conservatively estimated at 14,000 organisms, 12,400 were *Bact. coli* and, since many of the remaining organisms were *Streptococcus faecalis*, it seemed likely that this carton had been contaminated with faeces. The other coliform organisms listed in Table III were strains of *Achromobacter* isolated from two cartons and paracolon isolated from a single carton.

Five cartons contained more than 100 streptococci: in two cartons these were of "viridans" type, in two they were non-haemolytic, and in one they were *Str. faecalis*.

Other organisms found in large numbers were Gram-negative cocci, aerobic spore-bearing bacilli, and moulds. With one exception these all belonged to non-pathogenic species: the exception was that one carton gave an almost pure growth totalling 360 colonies of *Aspergillus fumigatus*.

An important organism not shown in Table III is *Proteus*. Sampling plates from three cartons obtained from the wards were overgrown with this organism. No *Proteus* was isolated from cartons obtained from the stores. From cartons obtained from the wards *Pseudomonas pyocyanea*, a member of the Friedländer-aerogenes group and a β -haemolytic streptococcus (which did not belong to Lancefield's Groups A, B, C, D, or G) were isolated from one carton each in very small numbers (less than 10 per carton).

The extent to which cartons were contaminated bore no obvious relation to the type of ward or their rate of turnover of cartons. In general the total counts were lowest in those wards, medical or surgical, where cartons were stored under conditions preventing free access of dust.

Support for the view that contamination of the cartons was usually airborne was given by the fact that the incidence of *Staph. aureus* in the flora of cartons agreed surprisingly well with what was already known of the incidence of this organism in the air of different wards. In particular, the air of two wards used for patients with skin diseases was usually far more heavily polluted with *Staph. aureus* than the air in any other ward in the hospital; these two skin wards were the main source of *Staph. aureus* from sputum cartons. The numbers of these organisms isolated from the two skin wards and 18 other wards are compared in Table IV. Of the nine cartons from the skin wards which grew *Staph. aureus*, four contained

TABLE IV
RECOVERY OF *STAPH. AUREUS* FROM ALL SOURCES

	Source of Cartons	
	Skin Wards	Other Wards
No. of cartons sampled	10	90
" " " yielding <i>Staph. aureus</i>	9	20
Total no. of organisms isolated . .	7,388	34,504
" " " <i>Staph. aureus</i>	2,864	478
<i>Staph. aureus</i> expressed as % of total organisms	38.8	1.4

No *Staph. aureus* were isolated from 100 cartons from the stores.

more than 100 of these organisms compared with only one of the 20 cartons from the other wards. No *Staph. aureus* was isolated from 100 cartons obtained from the stores.

Of the various organisms found in contaminated cartons it was thought that *Staph. aureus* and *Bact. coli* would be most likely to cause confusion in the examination of specimens of sputum. Experiments were therefore carried out with cartons which had been deliberately contaminated with dust containing these organisms.

Specimens of sputum arriving in the laboratory were cultured in the routine way and if no *Staph. aureus* or *Bact. coli* were found the specimens were divided into two portions: one portion was placed in a carton obtained from a fresh batch known to contain few or no organisms; the other portion was placed in a carton contaminated with 100–200 *Staph. aureus* or *Bact. coli*. The two cartons were then handed as unknowns to student technicians who were asked to culture the specimens on blood agar and report on the presence or absence of these two organisms. A similar procedure was used for specimens of saliva. The results of some of these investigations are shown in Table V.

In these and other experiments it was difficult to detect small numbers of *Staph. aureus*. *Bact. coli*, on the other hand, was recognized much more often since, unlike *Staph. aureus*, even a single colony of *Bact. coli* stands out in the area of heaviest inoculum. With specimens of saliva—and in this laboratory some 20% of sputum specimens consist of little else—small numbers of

TABLE V
RESULTS OF CULTURING SPECIMENS FROM CONTAMINATED CARTONS

	Sputum	Saliva
Specimens yielding <i>Staph. aureus</i> after collection in:		
(a) Clean cartons	0/24	0/12
(b) Cartons containing 100–200 <i>Staph. aureus</i>	2/24	0/12
Specimens yielding <i>Bact. coli</i> after collection in:		
(a) Clean cartons	0/12	2/12
(b) Cartons containing 100–200 <i>Bact. coli</i>	4/12	10/12

Bact. coli were recognized very easily. In normal circumstances an occasional colony of a coliform organism would be ignored. However, with three specimens of saliva and one specimen of sputum more than 10 colonies of *Bact. coli* were counted, and it is probable that in the ordinary way these organisms would have been investigated further.

Discussion

To assess the significance of organisms isolated from sputum is one of the most difficult problems in routine clinical bacteriology. In recent years the problem has become more difficult because the widespread use of powerful antibiotics has vastly increased the number of specimens containing *Bact. coli*, *Proteus*, paracolons, and other Gram-negative bacilli which are able to gain a footing in the respiratory tract when the normal coccal flora are suppressed. Although these coliform organisms are often present in only small numbers there are advantages in investigating them, as the findings may occasionally be of help if the same organisms are isolated from subsequent specimens from the same patient.

If attention is going to be paid to small numbers of coliform organisms it is reasonable to insist that the specimen should be collected in a clean container. Of all the organisms isolated from contaminated cartons it seems that coliform organisms are most likely to cause confusion. How often this occurs is largely irrelevant as the problem can be solved quite simply: sputum cartons should be individually wrapped, kept in a dust-proof container, or stored with their lids on.