Interenvironmental Transfer of Microorganisms on the Exterior Surfaces of Jet Aircraft

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The likelihood of microorganisms being transferred to new environments by jet aircraft was investigated. Initial random sampling of the aircraft surface revealed the presence of microorganisms in varying numbers on different aerodynamic surfaces. Bacteria of the genus Bacillus were the most common isolates, comprising approximately one-third of the total organisms found. The most frequently isolated fungi were Cladosporium, Alternaria, Penicillium, and several yeasts. Sampling of surfaces before and immediately after a flight demonstrated that microorganisms were collected during flight in areas protected from the airstream and lost in those areas directly exposed to it. These experiments also showed that the majority of the organisms contaminating the aircraft were acquired from the air at ground level. The placement of microorganisms on the aircraft surface before a flight and determination of their survival after flight indicated that the test organisms were most likely to be transported in the areas protected from the airstream. The organisms showing the best chance of being transferred seem to be the sporeforming bacteria, arthrospore-forming fungi, and some yeasts. All phases of this work showed that microorganisms could be carried by jet aircraft to environments they could not reach by natural means of dispersal.

During the last decade, the commercial use of jet aircraft has become increasingly common. These aircraft cover greater distances in shorter periods of time than ever before. If microorganisms are on the exterior surfaces of these aircraft and can remain viable during a flight, they could be transported to environments they would otherwise be unable to reach by natural means of dispersion. This study was undertaken to investigate the likelihood of long distance transport occurring on the exterior surfaces of jet aircraft. Those organisms carried on interior surfaces and by passengers were not within the realm of this study because the aircraft used were not in routine commercial service.

The initial phase of this research was intended to confirm the natural occurrence of viable microorganisms on the exterior surfaces of jet aircraft and to determine their distribution as to numbers and kinds on different areas of the aircraft surface. This involved the collection of many samples from a variety of areas on the surface of the aircraft. The second phase was directed toward a further investigation of both the source and method of contamination. Samples were collected from specific areas of the aircraft immediately after the flight. The final phase tested the ability of specific microorganisms to be transported on different areas of the aircraft surface. This involved the application of known numbers of organisms to specific surfaces before a flight and the determination after the flight of the number of viable organisms remaining.

MATERIALS AND METHODS

Aircraft samples. Samples were collected from four aircraft of the DC-8 Super 60 series of commercial jet transports (Douglas Aircraft Division, McDonnell-Douglas Corp., Long Beach, Calif.). Three of the four were undergoing flight testing during the period of this study. The fourth was in the final stages of construction prior to predelivery flight testing. The sampling of these aircraft was carried out at the Long Beach, Calif., plant of the Douglas Aircraft Division. Swab techniques were used to remove samples from the aircraft. Although other methods may give better results in specific areas, this proved to be the best single means of sampling the wide variety of surface configurations encountered on the aircraft. A template placed on the aircraft surface left a 10-cm² area exposed. The organisms were removed from this area with a swab carried to the aircraft in 2.0 ml of 0.1% peptone solution. The samples were refrigerated during transport to the laboratory for processing.
total time between taking a sample and its processing in the laboratory never exceeded 1 hr.

**Application of organisms.** Six different organisms were applied to the aircraft surface in the final stages of the project. On each flight, one organism was placed on four areas of the aircraft surface. The six organisms used were *Bacillus cereus*, *Pseudomonas aeruginosa*, *Rhodotorula* sp., *Cryptococcus diffluens*, *Chrysosporium* sp., and a *Coccidioides*-like organism.

The organisms were grown in the laboratory on appropriate media prior to placement on the aircraft surface. Trypticase Soy Agar (TSA; BBL) was used to grow the bacteria, and Sabouraud Agar (glucose, 40 g; peptone, 10 g; agar, 20 g; distilled water, 1,000 ml) was used for the fungi. As close as possible to the proposed departure time, the culture was prepared for application to the surface by suspending the growth from the plate media in 100 ml of 0.1% peptone. Before application of the culture from a spray bottle, the aircraft surface was cleaned to remove dirt and any contaminating organisms. The cleaning procedure consisted of spraying the surface, first with trichloroethylene, then with 70% ethanol, and finally with sterile distilled water. A control, consisting of the same organism placed on the surface of a coupon of aircraft-type aluminum alloy with the procedure employed for application to the aircraft surface, was included. This coupon was sampled at the same time and in the same manner as the areas on the aircraft. This control was kept on the ground for the duration of the flight and sampled again at the same time the after-flight samples were removed from the aircraft. This represents the number of organisms that would survive the flight period on an aluminum surface without flight being a factor. Before-flight samples were collected as close as possible to the actual take-off of the aircraft, usually less than 30 min before take-off. The areas were again sampled with the same swab procedure as soon as possible after the plane landed, usually within 15 min of landing.

**Laboratory procedures.** The processing of the swab samples in the laboratory was performed by standard procedures (1), and serial dilutions were plated on TSA and Sabouraud Agar. After appropriate incubation periods, the bacteria and fungi were isolated and identified. The fungi were identified to the genus level by the classification of Gilman (5). The yeasts were identified as far as possible on the basis of colonial and microscopic morphology as suggested by Lodder and van Rij (13). All bacteria were grouped by microscopic morphology and staining characteristics into four large groups: gram-positive spore-forming rods, gram-positive nonsporeforming rods, gram-positive cocci, and gram-negative rods. Identification of the genus and species of isolates from the samples taken before and after flight and those from a set of aluminum coupons placed near the aircraft was based on standard morphological and biochemical criteria (Bergery's Manual, 7th ed.).

**RESULTS**

**Initial sampling.** The initial sampling involved a total of 53 samples from different surface areas. These areas can be arranged into groups on the basis of their degree of exposure to the airstream. The configuration in some areas is such that during flight they are directly in the airstream. Areas of this type include the wings, flaps, many parts of the fuselage, and areas of the tail. Other areas, such as the front and rear landing gear, wheels and tires, and cargo storage areas, are almost completely protected from contact with the airstream. The third group includes those areas partially exposed to the airstream. These are the surfaces with shapes irregular enough to disrupt the airflow, and includes parts of the fuselage around doors and windows, air intakes, and any area in which there are sudden changes in surface shape.

The average numbers of microorganisms isolated from each of these area types are shown in Table 1. These counts are based on total numbers of microorganisms in the 10-cm² sample area. The averages for samples from aircraft that had never flown are also presented. These averages demonstrate considerable variation within each type area, but the pattern of increasing numbers of organisms with increasing protection from the airstream seems quite clear, whereas aircraft that have never flown show less variation from area to area.

The bacteria and fungi isolated from these initial samples are presented in Table 2. In all cases, the genus *Bacillus* was the predominant bacterial isolate. The other three groups show variation from area to area and relatively lower numbers. The fungi referred to as nonsporulating types were those that formed no recognizable sporulating structures on any of the media used. Many of these may be Ascomycetes.

| Table 1. Numbers of microorganisms found by random sampling of different surfaces |
|-----------------------------------|-----------------|-----------------|-----------------|
| Area type                         | No. of samples | Range of counts/cm² | Avg counts/cm² | New aircraft (counts/cm²) |
| Smooth surfaces                   | 11             | 1-42             | 13.5            | 21.0                  |
| Fuselage................................| 5              | 5-16             | 7.2             | 29.6                  |
| Wings, flaps, engines.............| 7              | 8-100            | 46.0            | 82.0                  |
| Irregular surfaces                | 6              | 7-136            | 48.8            |                      |
| Air intakes..............          | 4              | 10-322           | 111.7           |                      |
| Doors and windows.................| 9              | 19-146           | 66.0            | 26.5                  |
| Protected surfaces                | 2              | 142-984          | 613.0           | 40.0                  |


Before and after flight samples. The second phase of the project involved the sampling of areas of the aircraft surface immediately before a flight and the sampling of a closely adjacent area immediately after flight. The difference in numbers of organisms present in each pair of samples gives an indication of whether there is a gain or loss of organisms as a result of flight. The groups are arranged on the basis of their exposure to the airstream, as was done for the previous section. Table 3 presents the data grouped in this fashion. The bacterial species and fungal genera commonly found in the before- and after-flight samples are shown in Table 4.

### Table 2. Bacterial and fungal isolates from random samples

| Organisms                  | Smooth surfaces | Irregular surfaces | Protected surfaces | Never flown |
|----------------------------|-----------------|--------------------|--------------------|-------------|
| **Bacterial groups**       |                 |                    |                    |             |
| Gram-positive sporeforming | 36.8a           | 31.2               | 42.8               | 28.3        |
| rods                       |                 |                    |                    |             |
| Gram-positive non-          | 6.1             | 2.1                | 0.6                | 2.8         |
| sporeforming rods           |                 |                    |                    |             |
| Gram-positive cocci         | 3.1             | 8.8                | 3.7                | 1.0         |
| Gram-negative rods          | 7.1             | 2.1                | 3.7                | 2.8         |
| **Fungal genera**          |                 |                    |                    |             |
| Cladosporium               | 13.3            | 9.4                | 8.1                | 7.6         |
| Penicillium                | 3.1             | 10.4               | 5.6                | 4.7         |
| Alternaria                 | 7.2             | 9.4                | 6.8                | 6.6         |
| Yeast                      | 2.0             | 6.3                | 2.5                | 12.3        |
| Nonsporulating             | 4.1             | 4.2                | 7.5                | 2.8         |
| Rhodotorula                | 2.0             | 2.1                | 2.5                | 6.6         |
| Aspergillus                | 5.1             | 2.1                | 4.4                | 2.8         |
| Aureobasidium              | 3.1             | 1.0                | 8.5                |             |
| Fusarium                   | 2.0             | 1.0                | 4.4                | 1.0         |
| Epicoccum                  | 1.0             | 2.1                | 2.5                | 2.0         |
| Chaetomycetes              | 5.2             | 1.9                | 1.0                |             |
| Streptomyces               | 1.0             | 0.9                | 1.0                |             |
| Nigrospora                 | 1.0             | 0.9                | 1.0                |             |
| Others                     | 2.0             | 1.8                | 1.3                | 3.7         |
| **Fungi as per cent of**   |                 |                    |                    |             |
| total isolates             | 46.9            | 55.8               | 49.1               | 65.1        |
| Total no. of isolates      | 162             | 198                | 306                | 184         |

* Numbers represent the percentage of the total isolates in each area.

### Table 3. Average numbers of microorganisms on different type surfaces before and after flight

| Area type               | No. of samples | Before-flight organisms/cm² | After-flight organisms/cm² |
|-------------------------|----------------|------------------------------|----------------------------|
| Smooth surfaces         |                |                              |                            |
| Fuselage                | 6              | 17.3                         | 11.0                       |
| Wings, flaps, engines   | 4              | 6.0                          | 3.7                        |
| Irregular surfaces      |                |                              |                            |
| Air intakes             | 10             | 11.0                         | 19.0                       |
| Protected surfaces      |                |                              |                            |
| Wheels and tires        | 6              | 15.6                         | 80.6                       |
| Landing gear            | 14             | 44.1                         | 111.2                      |

* Numbers correspond to species numbers in Bergey's Manual (7th ed.).
TABLE 5. Numbers and types of organisms on aluminum coupons

| Coupon no. | Bacteria | Fungi | Total count |
|------------|----------|-------|-------------|
|            | Counts/cm² | Isolates | Counts/cm² | Isolates |             |
| 1          | 10       | Bacillus circulans  
             |          | B. cereus |          | 3           | Cladosporium  
             |          | Yeast     |          | Nonsporulating  
             |          | Aureobasidium |          | Ulocladium  
             |          | Nigrospora  |          |             |
| 2          | 14       | B. pumilus  
             |          | B. megaterium |          | 8           | Cladosporium  
             |          | Alternaria  |          | Yeast     |          | Nonsporulating  
             |          | Aspergillus  |          |             |
| 3          | 20       | B. pumilus  
             |          | B. circulans  
             |          | B. subtilis |          | B. megaterium  
             |          | Brevibacterium quae  
             |          | Micrococcus varians |          | 5           | Cladosporium  
             |          | Penicillium  |          | Alternaria |          | Nonsporulating  
             |          | Aspergillus  |          | Rhodotorula |          | Aspergillus  
             |          | Aureobasidium |          | Nigrospora  |          | Phoma     |

* Coupon one was placed on the roof of a maintenance building approximately 150 yards north of the aircraft parking area. Coupon two was placed on the roof of a flight test trailer about 100 yards north of the aircraft. Coupon three was on the roof of a trailer parked next to the aircraft.

Microorganisms were removed from the surface of the coupons with the same techniques employed for sampling the aircraft. The numbers and types of microbes isolated from these coupons are shown in Table 5. The results give an indication of the numbers and types that might find their way onto the aircraft surface and survive in a viable state.

Application of organism. As a preliminary to the actual application of organisms to the aircraft, the death rate of each test organism was determined. Cultures of each organism were applied to four aluminum coupons in the laboratory. The plates were sampled, and the samples were processed by the same procedure used for aircraft surface samples. The curves obtained for the two bacteria and four fungi are shown in Fig. 1. This figure demonstrates that, with the exception of the Coccidioides-like organism, the number of viable cells decreases rapidly during the first 2 hr. Even though the majority of the microorganisms fail to survive, in most cases a small percentage will retain viability for the full 6-hr period.

On each flight, organisms were applied to four areas of the aircraft surface. These four areas were selected on the basis of their exposure to the airstream. Area number one represented complete exposure to the airstream. Area two was a smooth airflow surface during most of the flight, but became irregular due to the turbulence created when the main landing gear was lowered. Area three represents direct exposure during take-off and landing and complete protection for the remainder of the flight. Area four is almost completely protected during the entire flight.

Each organism was applied to the four areas on the aircraft surface and the control surface, which remained on the ground, on each of two flights.
Cryptococcus diffluens was applied on three flights. Figure 2 presents the per cent survival of the six organisms. The percentages are adjusted so that the control done for each sample equals 100%. The duration of the flight for each sample is also included in Fig. 2.

**DISCUSSION**

This study has shown that there are many factors to be considered in assessing the relationship of a microorganism to the exterior surface of a jet aircraft and in understanding the chances of this microbe being carried by the aircraft to a new environment. The configuration of the aircraft surface that the microorganism contacts will have considerable influence upon its chance of remaining on the surface and being carried to a new environment in a viable state. All phases of the study indicate that as the exposure to the airstream decreases the number of organisms increases. The protected areas, therefore, appear to be those in which the microbes have the best chance of surviving the rigors of flight. In these areas, the organisms are not subjected to the harsh conditions encountered in the smooth and most of the irregular areas.

The way in which the organisms reach the surface will also affect the changes of successful transport. Many studies have shown that the numbers of microorganisms decrease as the altitude increases (3, 6, 15) as does the viability of the organisms present (3, 12, 14). At high altitudes and normal operating speeds, the jet aircraft would be likely to acquire very few microorganisms. Most of the organisms found must be the result of ground level contamination or those encountered when flying at very low altitudes. This is indicated by the relative percentages of bacterial and fungal isolates found in the random samples in which the percentages of the two types are nearly equal. Studies have shown that at low altitudes the proportions of bacteria and fungi are about equal (4), whereas at high altitudes the bacteria comprise as high as 90% of the microbial populations (3). It is significant that the contamination occurs at or near ground level because this is the area of maximal concentrations of microbial cells and also the times at which the protected areas are exposed.

The type of organism contacting the surface will also influence the likelihood of transfer. Sporeforming bacteria and some fungi appear to be the types best able to withstand flight. The methods used in this study limit the fungal isolates to those types that will grow on laboratory media. This makes it impossible to detect many of the rust and smut fungi that are commonly found in the air (2, 7–10). In some instances, these types constitute a majority of the organisms, especially during the night (7). The rust and smut fungi could be very significant if they were on the surface of the aircraft, since they are plant pathogens that depend on the wind for the dispersal of their spores (11).

If microorganisms are carried to new environments by the aircraft and arrive in a viable state, they must leave the aircraft surface and reach some nutrient environment. During the landing,
the landing gear is exposed to the airstream, at which time organisms could be blown off. Many microbes are picked up on wheels and tires during take-off. When the landing gear is retracted, the spinning wheels could throw organisms onto the large doors that enclose the landing gear. The organisms could be blown off of these doors when they are opened to lower the landing gear.

This study has indicated that the transport of microorganisms to new environments on the exterior surfaces of jet aircraft is possible and could result in the distribution of microbes to environments they probably could not reach by natural means.

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