Contamination of Stuffed Cloth Toys on a Pediatric Unit

Virginia Lois Fowler
Dynnette Nelson Hart

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CONTAMINATION OF STUFFED CLOTH TOYS
ON A PEDIATRIC UNIT

by
Virginia Lois Fowler
Dynnette Nelson Hart

A Thesis in Partial Fulfillment
of the Requirements for the Degree
Master of Science in the Field of Nursing

May 1968
Each person whose signature appears below certifies that he has read this thesis and that in his opinion it is adequate, in scope and quality, as a thesis for the degree of Master of Science.

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CHAPTER I

INTRODUCTION AND METHOD OF STUDY

I. INTRODUCTION TO THE STUDY

There is frequent concern over the problem of environmental contamination, particularly in an hospital environment. This is a valid concern since hospitals have patients who carry and shed pathogenic organisms.

Many hospitalized patients have a lowered resistance to infectious diseases. This is especially true in the pediatric unit since it may have patients with respiratory diseases, diabetes, those who have undergone surgery or those with long-term hospitalization. In addition children are more susceptible to many organisms than are adults.

"Infections are extremely frequent causes of concern during childhood, since the infant and the child do not have the immunity to many of them [the infectious organisms] as does the adult."¹ When the environment harbors potentially pathogenic organisms, there is a greater possibility that the child will acquire an infection.

In pediatrics, this potential hazard of environmental contamination is accentuated because habits and play patterns of the very young neither protect themselves nor their neighbors from the spread of potentially pathogenic organisms. Children are neither disciplined nor motivated to carry out the principles of hygiene, which

may include proper handling of respiratory secretions, washing of hands, and not sharing toys while ill.

II. THE PROBLEM

Children's lack of motivation and discipline to carry out good principles of hygiene leads to activities such as uncovered coughing, wiping nasal and oral secretions onto objects in the environment, handling of urinary and bowel excreta, insertion of objects, edible or nonedible into their mouth and lack of handwashing at appropriate times. These activities lead to dissemination of all types of organisms into the environment.

When a child is ill an increased number of pathogenic organisms, such as might come from sputum or excreta, are spread into the environment. Thus in hospitals the presence of ill children indicates the possibility of more pathogenic organisms being present in the environment.

One type of object frequently included in a child's play materials is stuffed toys. The play habits of a young child using a stuffed toy frequently include sucking and drooling on it, wiping his nose on it, and taking it with him wherever he goes. Toy contact with bowel and bladder excreta also frequently occurs.

Stuffed toys are usually found to be present on hospital pediatric wards, either as property of the hospital or the child's personal toy brought from home.2,3

3 Eva Noble, "The Value of Play for Young Children in Hospi-
Articles in journals of nursing have stated criteria for safe toys for hospital use. Safety includes rounded edges, no small detachable parts, nonallergenic material, and a safe finish.\textsuperscript{4,5} What constitutes a "safe finish" was not defined. A safe finish could mean many things, such as no toxic paint or dyes, presence of a bacteriocidal action, and an easily washed surface.

The writers' concern pertains to the microbial safety of cloth stuffed toys. Studies have been done of articles such as mattresses and bedding and these articles were found to be harborers of pathogenic bacteria. No studies were found to have been done regarding the possibility that stuffed toys also carry pathogenic bacteria. These toys differ from previously studied bedding in that they are not regularly changed and washed, they can be easily passed from child to child, and they come in frequent contact with the children's mouth, hands, diapers, and the floor. It is also difficult, if not impossible, to remove organisms from cloth stuffed toys by a simple wiping method. Research is needed as to the safety of cloth stuffed toys in regard to contamination with pathogenic organisms.

Purpose of the Study

The purpose of this study was to provide data for an evaluation of the role of toys as a means of disseminating organisms into


the environment. There were two parts to this study. The first was to determine if cloth stuffed toys used by children in a pediatric unit of a selected hospital could serve as carriers of bacterial pathogens.

The second part was to determine how long selected bacterial pathogens can survive on the same cloth stuffed toys as were used in the selected hospital.

Statement of the Problem

The problem investigated in this study was the possibility of cloth toys serving as carriers of bacterial pathogens.

Hypothesis

Potentially pathogenic organisms found on the throat culture of a hospitalized child will also be found on the toy the child uses, and, conversely, if no potential pathogens are found in the throat, none will be found on the toy.

Assumptions

1. The techniques for collecting and culturing the organisms from the cloth toys were assumed to be appropriate.

2. The procedures for isolating and identifying the organisms were assumed to be bacteriologically correct.

3. It was assumed that the researchers did not introduce pathogenic organisms onto the cultures.

4. It was assumed that the toy was used at some time by the child.
Limitations

1. The study was limited in that only one small part of the toy, four square inches on each side, was cultured at each time interval.

2. The toys were used in varying ways and for varying amounts of time by the different children.

Definitions

**Clean.** Absence of potential pathogens.

**Contamination.** The soiling of an object by potentially pathogenic organisms.

**Direct transfer.** Immediate transfer of organisms from one thing to another or one person to another without the intervention of subsidiary means.  

**Fomite.** Any substance or object other than food that may harbor and transmit infectious organisms.

**Indirect transfer.** Not immediate or straight transfer of organisms from one thing to another or one person to another, but through an intermediary agent.

**Pathogen.** "Any disease-producing microorganism."

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7Ibid., p. 521

8Ibid., p. 672.

9Ibid., p. 1007.
III. METHOD OF STUDY

A descriptive survey was the method of study chosen for the research done in the hospital. The exploratory method was used for the research done in the laboratory setting.

Permission to conduct this study was obtained from the Research Advisory Committee on Human Experimentation, the Director of Nursing Service, and the Chief of Pediatric Service.

The study was conducted from June 18, 1967, to August 3, 1967. A pilot study, described in Chapter III, was done previous to the hospital study.

Literature was reviewed for several purposes: (1) to identify adequate procedures for collecting and classifying the data; (2) to review current research on cloth fomites as harborers of pathogens; (3) to review current bacteriological knowledge regarding specific microorganisms (listed later) found in the hospital and used in the laboratory situation. Chapter I includes a review of the current literature pertaining to the specific microorganisms studied, including their characteristics and methods of analysis. The review of literature in Chapter II contains current research and opinions on methods of transfer of organisms and methods of collection of organisms from cloth.

In order to obtain a more complete picture of cloth toys as possible carriers of bacteria, the toys were studied in both the hospital and laboratory settings. In the hospital, research was done to determine the organisms present on a toy after use by a child, and to see if organisms present on the toys were the same as those found
in the child's throat at the time of admission.

In the laboratory, research was done to determine the length of time specific organisms would survive on the toys in a controlled situation.

The toys chosen for study were covered with medium weave cotton material. The stuffing was either nylon stockings or foam rubber. Like small pillows, the toys were in irregular shapes with no distinct arms or legs and no articles such as buttons sewn on the toy.*

All such toys from the pediatric unit were removed from the unit and machine washed in hot water with detergent by the researchers. Machine drying was found to be inadequate, so the toys were hung in the sun to be dried. This was the method of washing used by the unit whenever the toys were cleaned. After washing by the researchers, the toys were placed in new plastic bags. This process was carried out in order to minimize the number of organisms present on the toys when first given to the child, or before use in the laboratory.

Hospital Survey

Selection of sample. All children between the ages of six months and six years who were admitted to the pediatric unit of the selected hospital and whose hospitalization would be more than seventy-two hours were selected to receive a toy for study. Children within this age group who refused the toy were not included in this study.

*See Appendix.
It has been the observation of the researchers that children in the age group six months to six years use stuffed toys more commonly than older or younger children.

If it was known that a given patient's hospital stay would be less than seventy-two hours, he was not included in the study. Examples of such situations are patients scheduled to have tonsillectomies or some types of eye surgery.

**Sequence of events.** At the time of admission, a child who was chosen for this study received one of the selected toys. Prior to placing the toy in his bed, the front and the back of the toy were cultured, using two plates, as a control and baseline determination of organisms present. The toy was also marked with the child's name. One throat culture was obtained from the child.

Cultures were taken of the toy twenty-four, forty-eight, and seventy-two hours after the toy was given to the child. It was felt that periodic cultures gave a more complete picture of organisms present than if only one culture had been taken.

Before and after handling any of the toys used in this study the researchers washed their hands using an antibacterial soap and a one to two minute scrub. This was to reduce the possibility of transferring organisms through hand contact.

**Recording of information.** Record was kept of the child's age and the hospital diagnosis. A log was kept of the types of infections present in the unit, based on the laboratory reports on the charts.
Technique of culturing. The Rodac culture plate, as described in the review of literature, was chosen for obtaining cultures from the toys because of its unique construction which allows direct contact between the surface of the agar and the cloth. When taking a culture, the lid of the culture plate was removed; the agar surface in the plate was pressed flat against the toy until the plastic rim made contact and held there for two seconds; the cover was then replaced. It was imperative that the whole agar surface come in contact with the toy in order to be able to count the colonies per square inch of cultured surface.

Two cultures were taken at each culturing time, one from the front and one from the back of the toy, in order to increase the possibilities of obtaining a more representative sample of the organisms present on the toy.

The Rodac plates were prepared by using an automatic pipetting machine. The total volume of media in each plate was 15.5 milliliters. The bottom layer consisted of 7.75 milliliters of clear Difco tryptose blood agar base, while the top layer was 7.75 milliliters of the same blood agar base with fresh sheep blood added. When plates containing 15.5 milliliters of blood agar were tried, observation of the types of hemolysis was difficult. With the layer of clear agar base on the bottom, the visibility of hemolysis in the top layer of mixed blood and agar was much better.

Advisability for the use of blood agar is based on the fact that many of the common pathogenic organisms expected to be found on the toys grow better on blood agar; such organisms as hemolytic streptococci will not grow on plain agar.\textsuperscript{11}

In obtaining a throat culture, a dry, sterile swab was rubbed on the posterior pharyngeal wall, the tonsil, or tonsillar fossa, as indicated.\textsuperscript{12,13} The swab was immediately rolled over approximately one-third of the surface of the agar in a blood agar petri plate. Using a sterile loop, the inoculum was then streaked over the rest of the plate so as to obtain isolated discrete colonies for identification.\textsuperscript{14}

Throat cultures were used rather than nasal cultures, because this study was concerned with the variety of potentially pathogenic bacterial organisms present in the oropharyngeal cavity of the pediatric patients. Nasal cultures, compared to throat cultures, usually reveal more diptheroid bacilli and staphylococci and less streptococci, especially the potentially pathogenic types, and the Gram-negative cocci of the Neisseria types. The alpha hemolytic streptococci and Gram-negative cocci appear to constitute the basic normal flora

\begin{itemize}
  \item \textsuperscript{11}Erwin Neter and Dorothy Rae Edgewater, Medical Microbiology, fourth edition, Philadelphia: F. A. Davis Company, 1962, p. 27.
  \item \textsuperscript{12}Isabelle Gilbert Schaub and M. Kathleen Foley, Diagnostic Bacteriology, third edition, St. Louis: The C. V. Mosby Company, 1947, p. 71.
  \item \textsuperscript{13}Ernest Jawetz, \textit{et al.}, Review of Medical Microbiology, seventh edition, Los Altos, California: Lange Medical Publisher, 1966, p. 267.
  \item \textsuperscript{14}Ibid.
\end{itemize}
of the nasopharynx and oropharynx.\textsuperscript{15}

**Laboratory Study**

The purpose of the laboratory experiment was to determine the length of time the organisms would survive on a toy after one known exposure to a specified microorganism. This experiment was done by spraying a suspension of the selected organism onto a toy in a closed chamber and determining the survival through periodic culturing.

The stuffed cloth toy used was identical in description with those used in the hospital setting. Before testing, the toys were washed in the same manner as mentioned previously.

The chamber in which the toy was placed for spraying was a hard clear plastic box open on the bottom and one side. The bottom of the chamber was sealed with masking tape onto clean brown paper. A plastic bag was taped over the front in such a manner that while spraying the toy, the organisms were contained in the box. The toy was suspended from the top of the chamber. Two toys were tested at a time, with each toy in a separate chamber.

Each organism was grown at 37° C. for 24 hours in Difco Todd-Hewitt broth. The concentration of the organisms was determined by plate count using a standard dilution scheme in which a measured amount of broth culture was added to a sterile water blank and the plate count then used to determine the number of viable organisms in

This determination was necessary in order to calculate how many organisms per cc. were sprayed onto the toy, thus providing a basis for comparison with the number of cultured organisms found in saliva.

An undiluted broth suspension containing a pure culture of the organisms was put into an aerosol sprayer and then sprayed directly toward the toy. Front and back of the toy were each sprayed with two cc. of the broth suspension. The toys were marked off into eleven squares the size of the Rodac plates, and two sprays of solution were directed into each square.

The dilution of organisms and method of spraying was chosen on the basis of results from the pilot study explained in Chapter III.

The decision to use an undiluted broth suspension is supported by evidence that the estimated number of cultured organisms per milliliter of saliva is recorded as $6.3 \times 10^7$, according to Miles and Wilson. The same concentration of organisms found in the undiluted broth suspensions more approximates this number than when diluted suspensions are used.

It is known that saliva has growth-promoting substances for a variety of oral species of organisms. Broth, with its protein, meat extract, and salt ingredients, supports growth of organisms.

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17 Wilson and Miles, op. cit., p. 2462.
18 Ibid., p. 2463.
19 Kenneth L. Burdon, Textbook of Microbiology, fourth edition,
When organisms are in a broth suspension, a growth-promoting environment is provided which is more similar to saliva than is a sterile water suspension.

The toy remained suspended throughout the days of culturing to prevent removal of organisms by contact with other surfaces.

Rodac plate cultures were taken immediately after the spraying. Subsequent cultures were taken at one-half hour, one hour, three hours, six hours, twelve hours, twenty-four hours, and repeated every twenty-four hours until the organisms were no longer recovered. If organisms were still present after 120 hours, no further culturing was done.

Cultures from the front and back were taken at each time interval. Only one culture was taken from any one square. If a site had been cultured more than one time, the number of organisms at that site may have been reduced by removal onto the culture media.

Certain organisms were chosen for study because of their importance in pediatric infections. The following characteristics, taken from Wilson and Miles, show why these were considered significant for study, and how each one can be classified.

**Serratia marcescens.** For use in the pilot study, *Serratia marcescens* was chosen as the bacterial organism because of its ease of identification by red colonies and similarities to the growth patterns of the pathogenic organisms used.

The usual habitat of *Serratia marcescens* is water and air.

It is also found occasionally on food, such as bread, meat, milk, and potatoes.

Its morphology is Gram-negative rods, often oval or coccobacillary arranged singly or in groups.

On an agar plate after two days of incubation at 37° C. the colonies are round, 1-2 millimeters in diameter, low convex, smooth, and glistening. A brick red pigment is formed only in the presence of oxygen and at a suitable temperature. Most strains grow well at 30 to 37° C., however, the red pigment forms better at room temperature.

Serratia is only occasionally pathogenic to man. Rabinowitz and Schiffrin in 1952 as reported by Wilson and Miles described an outbreak of infection in a children's ward, including cases of wound infections, meningitis and septic arthritis due to this organism. However, the organism is generally considered to be nonpathogenic.

Staphylococcus aureus. This actual or potential pathogen is found in suppurative lesions of man, cattle, sheep, goats, and chickens. It is common in the human nose and on the skin.

Its microscopic morphology is spherical cells, and smears from cultures on solid media show the cocci arranged in grape-like clusters. It is nonmotile, Gram-positive, and nonacid fast.

After 24 hours of incubation at 37° C., it shows circular colonies, which are low convex, opaque, and usually of a golden color and having a smooth glistening surface and an entire edge. It

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20Ibid., p. 842.
is butyrous in consistency. Hemolysis and pigment production usually occur by twenty-four hours, but may not appear until several days after the specimens are planted on blood agar plates. This preferably occurs at room temperature. **Staphylococcus aureus** is generally considered pathogenic if it produces a pigment, coagulase, and hemolysis of blood.

The pathogenicity of **Staphylococcus aureus** has been quite extensively studied in the hospital setting. In Messinger's study, he found a predominance of **Staphylococcus aureus** even in a hospital non-epidemic situation.\(^{21}\) Yanis considered this organism to be responsible for more infections than any other single bacterium. She stated that it is the offending organism of most boils, wound infections, eye infections, and throat infections. It can also cause secondary invasion.\(^{22}\)

In discussing the factors that favor staphylococcal infections, Cluff felt that **Staphylococcus aureus** is of primary concern as a human pathogen.\(^{23}\)

A study was done by Hinton, Maltman, and Orr to determine to what extent natural air drying influences the ability of staphylococci to survive and to retain those characteristics which are associated


with the ability of the organism to cause disease. Glass tubing was inoculated with *Staphylococcus pyogenes* (aureus) and then placed in loosely plugged sterile test tubes to allow drying to take place at room temperature. The dried samples were reconstituted by pipetting 10 milliliters of fluid into the tube and thoroughly mixing. The data suggested that during the course of natural desiccation not only are the original numbers of staphylococci released into the environment progressively reduced, but also the surviving organisms tend to show the development of cell injury which may be interpreted to give an overall reduction in infective capacity. Because of this, they felt it seemed "possible that recently contaminated areas may be much more dangerous than an area in which the organisms have been resident for a long period even when the total bacterial population in the second area is greater."24

Selwyn, Maccabe, and Gould felt that a further explanation for the low overall incidence of infection, in spite of the enormous load of environmental contamination, was because *Staphylococcus aureus*, when dried on fomites, lost much of its virulence.25

Rountree did a study by depositing cultures on squares of cotton and storing them in a cupboard for twenty-four hours to dry.26

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26Phyllis M. Rountree, "The Effect of Desiccation on the
The strains that showed no significant loss of viability for the first twenty-four hours of storage proved to be the ones isolated from epidemics of infection in the hospital. She felt that this survival of *Staphylococcus aureus* allowed greater chances for the organisms to infect new hosts.\textsuperscript{27} Her study also showed that the death rate of organisms per day decreased when the relative humidity at which the fomites were stored was raised.\textsuperscript{28}

*Streptococcus pyogenes.* *Streptococcus pyogenes* is a spherical coccus often arranged in chains of varying length, but including as a rule ten or more cocci. It is Gram-positive, a nonsporeformer and nonmotile. The members of the chain are often arranged in pairs similar to diplococci and rod-like forms are occasionally seen.

On blood agar, after twenty-four hours of incubation at 37° C., the colonies are very small in diameter, slightly raised, circular, opaque, and with an entire margin. The optimal temperature for growth is 37° C.

*Streptococcus pyogenes* produces a variety of infections in man which can be generalized and/or local.

In a study by Rammelkamp and colleagues, it was shown that storing streptococci at room temperature reduces their infectivity without impairing their viability in culture. They felt this finding

\textsuperscript{27}Ibid., p. 271.

\textsuperscript{28}Ibid.

explained the paradox of an environment heavily contaminated with virulent organisms yet sometimes containing many unaffected patients.\textsuperscript{29} Wilson and Miles concurred stating that although \textit{Streptococcus pyogenes} tends to die out in subculture unless preserved under particularly favorable conditions, it will remain viable for a long time in the dry state.\textsuperscript{30}

\textbf{Diplococcus pneumonia}. The name "diplococcus" describes its microscopic morphology. The ovoid cocci are arranged in pairs or short chains. When they are in pairs, the adjacent ends of the cocci are usually bluntly rounded, the opposite ends are more acutely pointed. They are nonmotile, nonsporeformers and Gram-positive.

The optimal temperature for growth is 37° C. On solid media, small, raised, circular colonies grow with a smooth surface and an entire edge. The colonies often show flat and smooth surfaces with sharp and steeply raised edges. A circumferential ring may form.

The colonies on blood agar plates are surrounded by a zone of alpha-hemolysis. This is a greenish discoloration with partial lysis of the red corpuscles.

\textbf{Diplococcus pneumonia} (pneumococci) is a fairly delicate organism which autolyses, or dies very rapidly. Growth is inhibited by a concentration of 1/500,000 to 1/100,000 optochin.

These organisms are often normal inhabitants of the upper


\textsuperscript{30}Wilson and Miles, \textit{op. cit.}, p. 734.
respiratory tract but can cause lobar pneumonia, sinusitis, otitis, meningitis, and other infections in man.

Method of Analyzing Organisms

The following methods were used to analyze the organisms in both the laboratory and hospital situations. All inoculated culture plates were immediately returned to the laboratory and incubated at 37° C., the optimal temperature for growth of the organisms expected to be found in the study.  

After incubation for twenty-four to thirty-six hours, the culture plate was removed for colony counting and morphologic inspection. In studies reviewed in literature, the colonies were counted at such times as eighteen, twenty-four, forty-eight, or seventy-two hours after incubation. Since no uniform recommendations could be found, the time interval of twenty-four to thirty-six hours was chosen by the researchers on the suggestion of Charles Winter, Ph.D., chairman, Department of Microbiology, Loma Linda University.


Morphological description of each colony, both macroscopic and microscopic, was recorded on a mimeographed sheet by checking one or more of the following categories: (1) round, (2) irregular, (3) smooth, (4) rough, (5) raised, (6) flat, (7) glossy, (8) dull, (9) pigmented (color noted), (10) opaque, (11) translucent, (12) pinpoint (2 mm. in diameter), (13) other size, (14) hemolytic, (15) mold, (16) other characteristics not included in one of the other categories, (17) Gram stain reaction, (18) cocci, (19) rods, (20) chains, (21) diplococci, (22) cluster, (23) chicken wire, (24) spore forming.* The idea for this check sheet came from a study of Greene, et al., but the actual design and contents were determined by the researchers. The purpose of macroscopic and microscopic description was to assist in classifying the types of organisms.

In addition to the above general analysis of the organisms, specific tests for organisms were done when indicated. These were indicated if macroscopic and microscopic description of the organisms proved to be similar to that of known pathogens.

Coagulase is an enzyme produced by many pathogenic staphylococci which, in conjunction with certain serum factors, coagulates plasma. In the coagulase test, one drop of normal saline is placed on a glass slide and a suspected pathogenic colony is transferred from the culture plate into the normal saline. A drop of citrated

*See Appendix.

37V. W. Greene, et al., op. cit., p. 569.

sheep plasma is added and while the solution is stirred, observation is made for evidence of coagulation. All coagulase-positive staphylococci are considered potentially pathogenic for man.\textsuperscript{39}

The optochin test is useful for differentiating between pneumococci and streptococci, since all pneumococci are sensitive to optochin (ethylhydrocupreine). "Pneumococci are killed by a concentration of 1/500,000 to 1/100,000, whereas streptococci require one of 1/5,000 or stronger."\textsuperscript{40}

These were the specific tests used to further classify the organisms as pathogens.

\textsuperscript{39}Ibid.

\textsuperscript{40}Wilson and Miles, \textit{op. cit.}, p. 728.
CHAPTER II

LITERATURE REVIEW OF METHODS OF TRANSMISSION AND
METHODS OF COLLECTING ORGANISMS

An extensive review was made of the literature published from 1960 to 1967, and found in the Vernier Radcliffe Memorial Library. Studies were reviewed concerning contamination by pathogenic organisms of environmental objects similar in texture to the stuffed cloth toys such as blankets, sheets, pillows and mattresses.

Literature was reviewed for several purposes: (1) to learn of current research on cloth fomites as harborers of pathogens, (2) to identify adequate procedures for collecting and classifying the data, (3) to review current bacteriological knowledge regarding specific microorganisms found in the hospital and used in the laboratory situation. Literature concerning specific microorganisms was discussed in the previous chapter, while Chapter II contains literature on methods of transmission of microorganisms and methods of collecting organisms from cloth fomites.

Through a review of the literature it was found that the studies concerning the role of the hospital environment as a means of disseminating organisms deal with three aspects. The first one is a measure of the hospital environment in regard to the degree of contamination. The second is determination of the degree of contamination transferred from one source to another. The third aspect is concerned with the transmission of pathogens from a contaminated object or person to a human and subsequently causing an infection.
The following studies are divided into the three ways infections can be transmitted. The studies included in each section are then discussed in relation to the above-mentioned aspects.

I. METHODS OF TRANSMISSION

Transmission of infections in the hospital can occur in the following ways: (1) airborne, (2) direct contact, and (3) indirect contact, with certain subdivisions in some of these categories. Epidemiologists, however, disagree on the relative importance of the various routes of transmission.

Airborne Transmission

Airborne transmission of organisms, one of the major methods of cross-infection, has been studied and reported in literature to a large extent. This review pertains to the air transmission of pathogenic organisms and is limited to the contamination of fomites.

In Harper's review of literature, she refers to four studies (done by Young and Porter, Dunbar, Wolf and associates, and Colbeck) which measured the amount of air contamination resulting from the movement of contaminated bedding and/or the presence of an infected person. Their results showed that there is an increased amount of pathogens in the air because of these factors. Colbeck's results

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43 Mary Alice Harper, "A Study of Door Pulls on Isolation
showed, after studying the movement of bedclothes and the presence of an infected person, that the movement of bedclothes caused more air contamination than the presence of an infected person.

Riley and associates showed that air transmission of pathogens can cause infection when seventy-one guinea pigs became infected with tuberculosis after being continuously exposed to air contaminated by patients with active pulmonary tuberculosis.44

Walter describes a clothing-bedding cycle which involved contamination from contact, droplet, and pus onto clothing and bedding. From these contaminated articles dissemination of organism was spread to the air.45

These studies measured the degree of contamination found in air samples, showing how organisms can be present in the air following exposure to contaminated articles.

In relation to air transmission, Yanis states that the changing of cubicle curtains, window drapes, and bed linens can raise the air count of organisms.46 This was stated as an opinion and no research data was provided to validate it.


Indirect Transmission

If organisms from fomites such as linen can raise the air count, as previously mentioned, these fomites can also possibly serve as an intermediary agent in transferring organisms to people or other objects. The majority of studies reviewed were concerned with measuring the degree of contamination of a particular fomite. Studies have also been done in which the source of contamination was determined and the extent to which pathogens from the contaminated source were spread.

Linen. Although bedding was free from pathogenic organisms when placed on the bed, Selwyn found that within a few hours after the linen change the bedding, especially the sheets, yielded heavy cultures of pathogens. The pathogens on the bedding were the same strains as those found on swabs taken from the anterior nares and skin lesions of the patients on the ward. He also showed that 50 percent of the patients acquired infection while in the hospital, but he did not demonstrate the source of the infection. This study was based on a two-year study of microbiological cultures on three dermatological wards. It seems to show that pathogens from an infected person can be transmitted to linens which are in close contact with that person.

McDaniel, in her review of literature, reports a study done by Rubo in which he exposed woolen blankets and cotton sheets to the

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environment in the same ward for three days for the purpose of measuring the degree of contamination present on the linens and blankets. The surface contamination was greatest for the cotton textiles and thus Rubo concluded that cotton sheets, spreads, and pillowcases can play a very significant role in contact infections. He felt that wool blankets were relatively unimportant when compared to cotton linen. 48

The findings of these studies show that linens harbor bacteria, however the extent to which linens spread bacteria or cause infection in an host was not investigated. Smith's opinion is that there is no controversy over the fact that linens are a potential reservoir for bacteria. 49

Blankets. It was brought out in Harper's review of literature that "perhaps the most controversial area in the whole pathogenic cross-infection problem is the matter of blankets." 50 The issues behind the controversy are whether blankets harbor bacteria and if they do can the pathogens from the blankets be transferred to a human host thus causing disease.

Literature supporting the belief that blankets are important carriers and transmitters of pathogenic organisms is presented first.


50 Harper, op. cit., p. 10.
Chatterjee did a study in which a prevalent bacteriophage type of Staphylococcus aureus was recoverable from such sources as asymptomatic carriers, blankets, and air samples from wards and operation theatres. Hospital blankets were found to be the most potent source of the prevalent bacteriophage type which caused infection. She found the same phage type on the blankets as present in the human carriers. However, she did not study whether the organism was transferred from the blanket to the human or from the human carrier to the blanket.

An editorial in the British Medical Journal, reviewing studies in literature, states that "blankets on hospital beds contain and freely shed vast numbers of bacteria. Pathogenic streptococci and staphylococci from purulent lesions and symptomless sites are often among these." Caplan's study reviewed by Harper showed how a long-standing invasion of a surgical unit with Staphylococcus aureus, Proteus vulgaris, and Pseudomonas pyocyanea was decreased by regular disinfection of the blankets. After formalinization of all blankets was routinely used, there was not a single wound infection for three months. This implies that the contaminated blankets had some role to play in the development of wound infections. However, other environmental


53Harper, op. cit., p. 11.
factors and the personnel on the unit were not studied and these may also have played a role in the reduction of infection.

The following studies support the belief that blankets are carriers of pathogenic organisms, but consider it unlikely that contaminated blankets may cause infection. Hare and Cooke felt that the importance of blankets as reservoirs of staphylococci has been "greatly exaggerated." They concluded this after finding few Staphylococcus aureus organisms present on blankets used by eleven patients who had a staphylococcal infection.54

In Howe's research involving 234 cultures from sixty-two clean blankets placed on the unit for use, he found that initially none of the blankets were heavily infected, the majority of them yielding only one to ten colonies per sweep plate. By the end of an eight-week period of use 80 percent of the blankets were contaminated with Staphylococcus aureus. The majority were bacteriophage types 80/81. The degree of contamination had not progressed further after another month of use.55 "None of the patients contracted a staphylococcal infection that could be attributed to a blanket although they were by and large an elderly, debilitated group of people."56 Based on the methods used the interpretations made seem to be warranted.


56 Ibid., p. 631.
Caplan claimed that blankets were a very small factor in the acquisition of *Staphylococcus aureus* by hospital patients. He reached this conclusion after studying 224 patients over a cumulative period of 1511 weeks. Correlation was made between nasal and blanket cultures. There were only seventeen cases in which staphylococcus of a given sensitivity pattern was cultured from a patient's blanket before it was cultured from his nose. One hundred and eighty-six patients carried at some time *Staphylococcus aureus* in the anterior nares, but only on these seventeen occasions was it possible that a staphylococcus from a patient's blanket infected his nose. This suggests that such transmission is but a small factor in the acquisition of *Staphylococcus aureus* by hospitalized patients.\(^5\) The environment on the ward was not controlled except for air samples taken during the changing of the bed linen which showed the presence of staphylococci organisms in the air. The possibility of other objects, personnel on the ward, or air transmitting the organisms must be considered.

In a study done by Perry, *et al.*, blankets containing large numbers of streptococci deposited from nasal carriers were issued to one group of men while another group of men received freshly laundered blankets. Before the contaminated blanket was issued and once or twice weekly thereafter, cultures of each blanket were obtained. The majority of blankets harbored large numbers of streptococci at the time they were placed in the barracks and only a slight decrease was noted during the second week. The clean blankets were not cul-

tured before they were issued to the men. Oropharyngeal and nasal cultures were obtained daily throughout the study period. The streptococcic colonies were classified into types. From the study it appeared that only two infections could conceivably be related to the contaminated blankets and in these two instances it was possible that some other source other than the blankets caused the disease.\textsuperscript{58}

It can be seen from the above studies that there is quite a general agreement that blankets do harbor pathogenic bacteria, but evidence is not conclusive that pathogens from blankets can cause infection in an human host.

\textbf{Mattresses.} Studies regarding mattresses have been concerned with the degree of contamination on the mattress and the possibility of pathogens being transferred from individuals to the mattresses.

Winner, after culturing an unstated number of mattresses, reported that they were "potentially dangerous harbourers of such bacteria as staphylococci, streptococci, \textit{Proteus}, \textit{Pseudomonas pyocyanea}, spore-forming organisms and tubercle bacilli."\textsuperscript{59}

In Harper's review of literature she noted that patients occupying certain rooms appeared to have more boils and similar infections. This infective process ceased after disinfection of the


mattresses and blankets. Also, repeated random cultures of mattresses were done and were found to harbor large numbers of bacteria. It was concluded that pathogens could pass through the sheets to the mattress and vice versa.\textsuperscript{60} Determination of the source of infection was not done and this conclusion was drawn without adequate data. Differentiation between blanket and mattress contamination was also not done.

The type of material used in the mattress has an effect on the organisms it grows. Foam rubber seems to have a bacteriocidal action. This action is especially effective in the presence of moisture. This was concluded after an unsuccessful attempt at the University of Saskatchewan to grow \textit{Staphylococcus aureus} in foam rubber.\textsuperscript{61}

In Howe's study plastic covered mattresses were not found to be an important reservoir of \textit{Staphylococcus aureus}. To reach this conclusion ninety cultures were taken from the mattresses of twenty-seven patients with lesions draining \textit{Staphylococcus aureus}. In only one case was the patient's mattress contaminated by the same organism as present in the lesion, but the plastic mattress covers were contaminated by the organisms in five of the fifty-two cultures. In continuation of this study, 512 cultures on seventy-three mattresses on one surgical ward over a three-month period were taken and these yielded only two cultures positive for \textit{Staphylococcus aureus}.\textsuperscript{62}

\begin{footnotes}
\item[60] Harper, \textit{op. cit.}, p. 15.
\item[61] \textit{Ibid.}, p. 16.
\item[62] Howe, \textit{op. cit.}, p. 630.
\end{footnotes}
Wickens' opinion is that mattresses play an important part in infection. He feels that the bellows effect of the interior springs soon forces bacteria to the surface. No study was done to validate his opinion.

The findings of these studies seem to indicate that mattresses can be potential harborers of pathogens. However, their role as to transmission of pathogens is unclear.

**Pillows.** In Harper's review of the subject of pillows it was stated that since a "considerable percentage" of patients become carriers of pathogenic organisms it is reasonable to assume that patients' pillows will eventually become infected. "After pillows are infected, they expel the pathogens every time the patient moves his head and every time the nurse fluffs the pillow for him." This was an opinion given without research validation.

**Curtains.** Rountree, in studying new cotton curtains on a new ward, found that the curtains could be contaminated with staphylococci present in the ward. Twenty-one percent of the curtains were found to release these organisms onto contact plates. They concluded that ward curtains should be regarded as potential trappers and dispersers of staphylococci.

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Direct Transmission

Direct transmission involves being transferred by a human host. Several studies have been done concerning the importance of fomites as carriers of pathogens in comparison to human transmission.

Rammelkamp, et al., showed that dust collected from the floors of barracks where epidemics were in progress and containing a large number of Group A streptococci failed to produce infection after inoculation into man. When fresh oropharyngeal secretions from a patient with a streptococcal infection were placed in the upper respiratory tract, an infection occurred; but once the secretions were dry, no infection occurred. They concluded that the main route of infection with Group A streptococci is by direct or intimate contact with an infected individual. Few, if any, respiratory infections were caused by the airborne route or by contact with environmental deposits.66

Rammelkamp, et al., also studied the transmission of staphylococcal infections. Studies of organisms from naturally contaminated diapers and blankets, such as might involve a carrier nurse or laundry attendant during routine duties, usually failed to result in colonization of the wound, skin, or respiratory tract. The average nasal carrier expels few organisms into the air; more of the staphylococci are removed from the respiratory tract by direct transfer from the hands to other areas of the body. They felt that

contaminated bedding and clothes do not appear to be a major source of infection. Rather, the most efficient method of transmission, when the organisms are carried in the nose, is by transfer to the hands, and, finally to the patient.  

An editorial in the Scottish Medical Journal, which reviewed studies in literature, agreed with the above studies stating that cross-infection seems to depend on direct transfer from the human host and to a lesser extent upon transfer from contaminants in the environment.

Rountree expressed the opinion that it is very difficult to incriminate such articles as ward textiles as the direct causes of sepsis. "One tenable view is that they merely reflect the degree of sepsis in a given place and that other human factors are responsible for the transfer of staphylococci to wounds."

It was an opinion of Leod that the presence of staphylococci in the air and on surfaces has been given undue attention as a means of cross-infection. The presence and numbers of the microbes in the air or in floor dust should indicate that there are staphylococcal infections in the neighborhood and not that bacteria in the air cause cross-infection. The likelihood that infection is transferred directly from person to person seems greater than transfer through the

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67 Ibid.


69 Ibid.
air in droplet nuclei or on dust particles.\textsuperscript{70}

Lt. Colonel Cooch has the following opinion regarding the various roles of cross infection: "The great divergence of opinion as to the relative frequency of air-borne spread, of direct contact and of fomites is probably not solely the result of prejudice and intransigence but more probably stems from the fact that in various situations each method plays a role which varies with the situation."\textsuperscript{71}

It also seems that the divergent conclusions of studies regarding the various aspects of contamination are due to differences in methods of research, and what factors are being studied.

II. METHODS FOR COLLECTING ORGANISMS

In repeated studies, various methods have been used to collect cultures of microorganisms from humans and cloth fomites. Recent and pertinent methods are reviewed in this section for purposes of substantiating the methods used in this study.

Culturing of Nose and Throat

After a survey of the literature it seems apparent that either throat or nasal swabbing can be used to obtain bacterial cultures, depending on the type of organisms that are being investigated.

In studies involving \textit{Staphylococcus aureus} the anterior nares


are often swabbed for culturing microorganisms.\textsuperscript{72,73,74,75} The nares were used because they proved to be the sites most frequently colonized by staphylococci in adults and pediatric patients.

\textit{Staphylococcus aureus} is included as a frequent inhabitant of the nasal passages in perhaps a third or more of healthy adults in the general population.\textsuperscript{76} "The flora of the nose consists of prominent corynebacteria, white and yellow staphylococci, and streptococci."\textsuperscript{77}

In contrast to the nares, the normal throat flora consists of alpha-hemolytic streptococci, aerobic and anaerobic staphylococci, gram-negative diplococci, diphtheroids, and occasionally lactobacilli.\textsuperscript{78,79}

Under the influence of weather and other factors that affect the mucous membranes, the composition of the flora in any one person's

\begin{itemize}
  \item \textsuperscript{72}E. Joan Stokes, \textit{et al.}, "Control of Hospital Staphylococci," \textit{Lancet}, 2:197-201, July 31, 1965.
  \item \textsuperscript{74}D. W. Bethune, \textit{et al.}, "Dispersal of \textit{Staphylococcus Aureus} by Patients and Surgical Staff," \textit{Lancet}, 1:480-483, February 27, 1965.
  \item \textsuperscript{78}Ibid.
  \item \textsuperscript{79}Burdon, \textit{op. cit.}, p. 329.
\end{itemize}
throat varies from time to time. The predominant varieties of bacteria at any particular time are likely to be the same as those that are most abundant in the throats of intimate associates at that time. The species that are dominant will differ from place to place and from season to season. "Many of these organisms found normally in the pharynx, such as most of the Gram-negative diplococci, are entirely harmless, while others, like the streptococci, are at least potentially pathogenic."80

**Streptococcus pyogenes** Group A are not commonly cultured from the healthy throat. However, these streptococci and other pathogenic microorganisms may occasionally be found in the pharynx of healthy carriers.81

**Culturing of Cloth Fomites**

In her study of fomentation covers, McDaniel used the triple contact plate method. She based her choice on the experience of Rubo and Dixon with this method. After stretching the fabric over an aluminum disc which was mounted on a wooden handle, a petri dish containing agar medium was pressed against the cloth. This was performed on three sites.82

In addition to the triple contact plate method, McDaniel describes the following two methods:

(1) the sweep-plate technique, in which the edges of an open petri dish containing medium were brushed several times

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81 Ibid.

across the fabric and (2) a percussion-plate technique, in which the fabric was stretched and clamped 5 centimeters above an exposed medium dish in a confined space and then struck with a steel ball.\textsuperscript{83}

Recently many objects in the hospital environment, including cloth articles, have been tested with the Rodac culture plate, a disposable direct contact plate. It consists of a specially designed plastic dish filled to the brim with agar medium making a slightly projected contacting surface of four square inches. On the bottom of the dish grid lines facilitate the counting of colonies. The plastic cover rests on a base at the outer edge of the dish, keeping it away from the culture medium.\textsuperscript{84}

The technique of sampling consists of removing the cover and placing the culture medium flat against a flat surface of the object being tested. Pressure is then placed on the plate until the rim makes contact. Pressure against the back brings any uneven areas of the medium or sampled surface into contact. The medium surface is flexible enough to allow use on concave surfaces with radii as small as eight inches. The plate can easily be rolled over convex surfaces.\textsuperscript{85}

Of the four above-mentioned methods for culturing cloth fomites, two employ direct contact. The Rodac culture plate appears to be the simplest method, and the only one readily usable for cloth stuffed toys.

\textsuperscript{83}Ibid., p. 8.


\textsuperscript{85}Ibid., p. 1023.
CHAPTER III

COLLECTION, PRESENTATION, AND INTERPRETATION OF DATA

I. HOSPITAL PILOT STUDY

The pilot study was conducted to determine if organisms could be isolated directly from the surface of the toy by the Rodac culture plate method, and what bacterial organisms could be isolated from the toys used by children in the hospital.

On April 20, 1967, a convenience sample of eight toys was selected for the pilot study. One culture was taken from each toy. No criteria were established for the selection of the cloth toys. Multiple types of cloth surfaces were sampled, such as smooth cotton and synthetic furs, since both hospital and personally owned toys were used. The age range of the children using the toys was five months to fifteen years.

From the growth of colonies on the blood agar plates, it was found that the organisms could be isolated from the surface of the toys by using the Rodac culture plate method. The plates were incubated and the organisms identified and examined according to the method described in Chapter I. The organisms found on the culture plates are listed as follows: Diplococcus pneumonia, Staphylococcus epidermidis, Streptococcus pyogenes, other Gram-positive cocci and rods, and Gram-negative cocci. The potentially pathogenic organisms found were Diplococcus pneumonia and Streptococcus pyogenes.

Although there was no observable difference in the number and type of organisms found on the different cloth surfaces, it was de-
cided for purposes of consistency and control to use toys made from
the same fabric.

One culture per toy was not considered an adequate sample
from the surface of the toys. Sampling of the total surface of the
toy was not feasible; therefore, it was decided to culture two sites,
one on the front and one on the back of the toy.

II. HOSPITAL COLLECTION, PRESENTATION, AND
INTERPRETATION OF DATA

Between June 18, 1967, and July 7, 1967, data was collected
in the pediatric unit of the old Loma Linda University Hospital.
During this time, twelve patients with the specified toys were
studied. On July 9 all patients were transferred from the old hos-
pital to the newly constructed Loma Linda University Hospital. From
July 9, 1967, until August 3, 1967, collection of data was continued
in the pediatric unit of the new hospital. This change was not con-
sidered cause to discontinue the data collection. During the time a
toy was being used by a child only the toy was cultured each twenty-
four hours. Cultures of the environment surrounding the child were
not taken.

Although the effect of the change in environment was not con-
sidered, the same number of patients (twelve) were studied in each
hospital and the data was temporarily kept separate. This was done
so that any difference in the results could be observed. Since
there was no observable difference, the data was combined for anal-
ysis.
The total sample consisted of twenty-four patients with their toys. The cases studied varied in age and clinical diagnosis. A hospitalization of 72 hours was considered necessary in order to adequately study the presence of microorganisms on the toys; therefore, patients who were not expected to remain in the hospital for this length of time were excluded from the study. Examples of this were those patients having tonsillectomies or certain types of eye surgery. The resulting sample consisted of twenty-one medical and three surgical patients. One half of the medical patients had diseases of an infectious nature, such as pneumonia, bronchitis, urinary tract infection, and cellulitis. The log kept of the types of infections present on the unit did not seem to provide any significant information useful in this study, so it was not used.

The ages of the patients ranged from five months to eleven years. The rationale for using one patient who was five months old was based on her physical and motor development which was similar to a six-month-old and also her desire to have a toy. The eleven-year-old was a mentally retarded girl who was willing to use a toy. The children were divided into age groupings to determine if the age of the patient would affect the presence or absence of potentially pathogenic organisms in the throat or on the toy. The way a ten-month-old child uses a toy differs from the way a six-year-old uses his toy. Because of this difference the age groups were arranged into those groups in which cloth toys would be used in a similar manner. From about five to eighteen months, infants frequently have oral contact with their toys. Ages two to three still
use cloth toys, but with less oral contact. From age four and upward there is a decline in the use of cloth stuffed toys. Some children reject them, while others still actively use them. There is minimal oral contact in this age group. As with any classification of children, the dividing lines of age groups are never as precise as stated.

Eight patients were between ages five to fifteen months, nine patients were between two and three years, six were ages five and six years, and there was one eleven years old.

One throat culture was taken of each child on admission. Before giving a toy to the child, control cultures, using the Rodac culture plate, were taken of the front and back of a toy which had been machine washed and hung in the sun to dry. The researchers' hands were washed before and after handling the toy. Cultures were subsequently obtained from the surfaces of the toy after twenty-four, forty-eight, and seventy-two hours of use.

The Rodac plate cultures were incubated at 37° C. for twenty-four to thirty-six hours, after which they were macroscopically and microscopically examined for colonies of bacterial growth. Colonies considered as potentially pathogenic bacteria were picked for pure culture and specific tests were carried out to identify the organisms. Records and gram-stain slides were kept of the organisms found on each patient and toy.

The results of the control cultures taken from the toy before giving it to the child showed no potentially pathogenic organisms. Therefore, pathogens later cultured from the toy were not introduced
by the researchers or former users of the toy. The washing and drying seemed to adequately destroy any pathogens which had been previously transferred to the toy.

Table I shows the number of cases in which the throat and toy cultures agree or disagree. Of the twenty-four patients studied, eight, or 33 percent, had the same potential pathogen(s) cultured from their toys as cultured from their throats. With six of these eight cases, *Diplococcus pneumonia* was the pathogenic organism found; one had *Staphylococcus aureus*; and in one case both organisms were present. The time intervals at which the potential pathogens were found varied between twenty-four, forty-eight, and seventy-two hours. There was no increase of contamination with pathogens over a period of use.

Although there were eight cases, 33 percent, in which the same organisms were found in both the patient's throat and on his toy, it would be suspected, but cannot be positively concluded, that the pathogens found on the toys came directly from the child using the toy. Since the study was conducted in the natural hospital setting, no attempt was made to prevent others from handling the toy and no cultures were taken of the environment. Bacteriophage typing was not done to determine if the strains were the same.

Besides the eight mentioned in the previous paragraph, there were four cases from which *Staphylococcus aureus* was cultured from the toys but not from the throat. This *Staphylococcus aureus* found on the toy may have possibly been from the child's nose which was not cultured, or from the environment, or from some other human carrier. As mentioned in Chapter I, *Staphylococcus aureus* is usually
TABLE I

NUMBER OF CASES SHOWING POTENTIAL PATHOGENS IN THE THROAT AND/OR ON THE TOY

<table>
<thead>
<tr>
<th>Pathogens Present on the Toy</th>
<th>Pathogens not Present on the Toy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens Present in the Throat</td>
<td>8</td>
</tr>
<tr>
<td>Pathogens not Present in the Throat</td>
<td>4</td>
</tr>
</tbody>
</table>

N = 24
more prevalent in the nose than in the throat.

Pathogens were not cultured from four, or 17 percent, of the toys used by children who had pathogens cultured from their throats. It is possible that the organisms would have been found if the toy had been cultured at a different time or site. Again, the toy may have been contaminated with pathogenic organisms, but due to death of the organisms, none were cultured. For example, a toy which had been handled by a child in the morning could have been left unused for the remainder of the day. If then the toy was cultured late in the evening, organisms of short viability, e.g. *Diplococcus pneumoniae*, would not have been isolated from the toy. Also, potential pathogens may have been present on one area of the toy, but not on the site which was cultured. Another possibility is that no organisms may have been transferred to the toy due to lack of use.

Seven of the eight cases in which no pathogenic organisms were cultured from the throat or from the toy were not diagnosed as having an infective disease. One of the eight cases was a patient with cellulites below the right eye. It was expected that potential pathogens would be cultured from the toy. The reason that they were not found may be that the toy was seldom used—a fact observed by the researchers.

Table II shows the relationship between the presence of potential pathogens and patient age groups. From this data it appears that half of the cases in the five to fifteen-month-age group, three of the nine patients in the two to three-year-old group, and one of the seven in the older age group had the same pathogenic organisms
TABLE II
NUMBER OF CASES SHOWING POTENTIAL PATHOGENS IN THE THROAT AND/OR ON THE TOY ACCORDING TO AGE GROUPS

<table>
<thead>
<tr>
<th>Ages</th>
<th>Same Pathogenic Organisms on Throat and Toy</th>
<th>Pathogenic Organisms in Throat Only</th>
<th>Pathogenic Organisms on Toy Only</th>
<th>None Present on Throat or Toy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15 months</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>2-3 years</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Over 3 years</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>
on their toy and in their throat. This loose inverse correlation with age may be based upon the well-known habit of the very young to have mouth-toy contact resulting in transfer of organisms from the throat to the toy.

When the percentage of positive correlation with the hypothesis is determined as shown in Figure 1, the inverse correlation with age is again shown. The reduced mouth-toy contact as a child increases in age has been observed by the researchers and also others when studying growth and development of children. This loose correlation also seems to support this observation.

No significant implications can be made from the frequency of distribution of cases by age grouping where organisms were found on the toy and not in the throat, or where there were no organisms found on either the toy or in the patient's throat.

Observation of factors which may have been responsible for the number of organisms cultured from the toy and which were not controlled are included because of the possible implications. How toys were used in the hospital may have affected the results of this study. In a few instances where the researchers observed a child using the toy just prior to culturing, more organisms were found on the culture plate. Also, in the two cases where a toy was being used in a croupette, more organisms were cultured from the toy. This corresponds with the finding that the death rate of organisms decreases as the relative humidity is raised as reported in a study by Rountree. These two cases were in the five to fifteen-month-age group, which may have also influenced the type of use and number of organisms cultured from the toy.
**FIGURE 1**

PERCENTAGE OF CASES ACCORDING TO AGE GROUPS SHOWING POSITIVE CORRELATION TO THE STATED HYPOTHESIS.*

*Hypothesis: Potentially pathogenic organisms found on the throat culture of a hospitalized child will also be found on the toy the child uses, and, conversely, if no potential pathogens are found in the throat, none will be found on the toy.
FIGURE 1

PERCENTAGE OF CASES ACCORDING TO AGE GROUPS SHOWING POSITIVE CORRELATION TO THE STATED HYPOTHESIS.*

*Hypothesis: Potentially pathogenic organisms found on the throat culture of a hospitalized child will also be found on the toy the child uses, and, conversely, if no potential pathogens are found in the throat, none will be found on the toy.
The fact that the children sometimes used other toys besides the toy given them by the researchers and nursing personnel sometimes removed the toy from the child's bed and placed it on a storage shelf for an indefinite length of time may explain why a small number of organisms were cultured from certain toys.

In a few instances the toy was found on the floor which could have decreased or increased the number of organisms cultured.

The shape of the toy may have affected the amount of use by the child. Having no distinctive features or appendages, this toy may not have been attractive to a child, especially an older child who may desire a realistic and useful toy.

From the data collected in the hospital it can be seen that toys are harborers of pathogens and potential pathogens can be transferred from a child's throat to his toy.

III. LABORATORY PILOT STUDY

The laboratory study was done to determine how long selected bacterial pathogens can survive on the same cloth stuffed toys as used in the hospital study. The pilot study was conducted to determine what concentration of organisms in the broth culture would be similar to the concentration of organisms in saliva, how to artificially contaminate the toy with the organisms, and a systematic method of culturing the organisms from the toy. Serratia marcescens, because of its characteristics as described in Chapter I, was the organism selected for this study.

The organisms, diluted in sterile water to a concentration of
4,000 organisms per cc., was sprayed onto the toy, using a rubber bulb, hand-operated atomizer. Twelve strong sprays were directed toward the top of the chamber. Results from cultures taken at predetermined times showed that there were from zero to twenty-five colonies present at the culture sites taken immediately after spraying. After three hours there were no living organisms cultured from the toy. These results appeared inconsistent with the findings of a study done by Fowler.86 After swabbing Serratia marcescens onto metal tables, she took cultures at determined intervals up to twenty-four hours. Serratia marcescens was still found surviving on the tables at twenty-four hours.

Further literature reviewed indicated that both the sterile water suspension and the number of organisms per cc. were unrealistic. Sterile water does not contain the protective and growth-supporting substances that are present in saliva; however, a Difco Todd-Hewitt broth solution might provide this environment. To more closely equal the number of organisms per cc. of saliva, it was decided to use an undiluted solution containing approximately 6.3 x 10^7 organisms per cc.87

To standardize the application of the organisms, eleven squares were marked off on the front and back of the two toys used in the study and two sprays were directed toward each square at a distance of six to eight inches. This also provided a systematic way of

86 Virginia L. Fowler, "Nursing Hypothesis on the Cleaning of Overbed Tables" (Nursing Hypothesis for Loma Linda University, 1966).
87 Wilson and Miles, op. cit., p. 2464.
culturing the toys, using a different square at each culture time.

The second trial using *Serratia marcescens* incorporated the changes mentioned above. An undiluted broth suspension, at a concentration of \(8.0 \times 10^8\) as determined by standard plate count method, was sprayed onto the toys. Figure 2 shows a graph of results of this study. In cultures taken immediately and thirty minutes after spraying, the colonies were too numerous to count; however, the general appearance of the culture plates taken at thirty minutes was less diffuse than the one immediately after spraying. There was a steady decline in colonies per culture plate until at twenty-four hours there was a mean of 41 colonies, and at forty-eight hours there was a mean of .75 colonies. Although at seventy-two hours no colonies were found, at ninety-six hours there was again a mean of .75 colonies. After 120 hours no colonies were cultured from the toy. The mean was the average of the total colonies on the four culture plates taken at each time interval. Also shown in each figure is the standard error of the mean, given at each culturing time.

Based on the results of the pilot study, this method of dilution and spraying of the pathogenic organisms was considered appropriate.

IV. LABORATORY COLLECTION, PRESENTATION, AND INTERPRETATION OF DATA

After suspending two toys from the top of two hard plastic chambers, they were sprayed with an undiluted broth suspension, con-
FIGURE 2
MEAN NUMBER OF COLONIES OF SERRATIA MARCESCENS
ISOLATED FROM CLOTH STUFFED TOYS,
0 TO 120 HOURS AFTER
CONTAMINATION
X ± SE
taining approximately $6.3 \times 10^7$, of one of the organisms selected for study. Immediately after spraying, Rodac plate cultures were taken from one space on the front and one on the back of each of the toys, using a different square at each time interval, and again at thirty minutes, one hour, three hours, six hours, twelve hours, twenty-four hours, and then at twenty-four hour intervals through day five. If before day five there were no organisms found from two consecutive culturing intervals, no further cultures were taken.

Four sites were considered adequate since the pilot study showed that the results from the four cultures were generally consistent. These four sites also increased the reliability of the results. The two toys were washed and sundried after each experiment was completed.

The culture plates were incubated at 37° C. for twenty-four to thirty-six hours, at which time the colonies were counted. A few organisms other than the specific organisms sprayed on the toy were also found on the culture plate, but these colonies were not counted. These colonies were considered to be contaminants from the environment. Although neither the toys nor the environment were sterile, no potential pathogens were ever found on the toys other than those sprayed onto them. It is unlikely that any of the pathogenic organisms studied were from any source other than spraying, since when spraying pneumococci no staphylococci were found, etc.

The types of organisms selected for study varied in the length of time they survived on the surface of toys. The following tables and explanations show the rate of survival of organisms.
The mean of the colony counts of the four sites was determined and plotted on semilogarithm graphs. The total range, showing the number of colonies found, is given on the graph at each time interval.

_Staphylococcus aureus_, in a concentration of $5.5 \times 10^8$ organisms per cc., was still found surviving on the toy at the end of 120 hours. However, the total number of colonies dropped from being "too numerous to count" (in the thousands) immediately after spraying to one or two colonies (a mean of .75 colonies per plate) per culture plate taken five days after the toy was sprayed.

By six hours the colony counts had dropped to a mean of 720. There was an unexplained rise to 1425 at twelve hours. The rise in count may have been due to inconsistencies in spraying. After the rise at twelve hours the colony count decreased until there was a mean of .75 colonies per plate at 120 hours. Figure 3 graphically shows the results.

_Streptococcus pyogenes_, a beta hemolytic streptococcus, was sprayed onto the toy using a concentration of $3.0 \times 10^6$ organisms per cc. On the immediate culture plates a mean of 538 colonies was found. As shown on Figure 4, the colony counts rapidly decreased until at twelve hours the mean count was 11 colonies per plate. Both the seventy-two-hour and the ninety-six-hour cultures showed that no organisms could be cultured from the toy.

_Diplococcus pneumonia_, in a concentration of $5.2 \times 10^6$ organisms per cc., was sprayed directly onto the toy. As shown in Figure 5, the mean colony count of the initial culture was 165.
FIGURE 3
MEAN NUMBER OF COLONIES OF STAPHYLOCOCCUS AUREUS
ISOLATED FROM CLOTH STUFFED TOYS,
0 TO 120 HOURS AFTER
CONTAMINATION
× ± SE
FIGURE 4

MEAN NUMBER OF COLONIES OF STREPTOCOCCUS PYOGENES ISOLATED FROM CLOTH STUFFED TOYS, 0 TO 96 HOURS AFTER CONTAMINATION

$X \pm SE$
FIGURE 5

MEAN NUMBER OF COLONIES OF DIPLOCYCUS PNEUMONIA
ISOLATED FROM CLOTH STUFFED TOYS,
0 TO 24 HOURS AFTER
CONTAMINATION
X ± SE
colonies. It decreased rapidly to 30 colonies at thirty minutes and 16 colonies at one hour. By three hours no colonies were found on the culture plates. Cultures taken at six hours and twenty-four hours also showed no colonies.

Thus, from the above data the survival rate of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Diplococcus pneumonia* is presented in Figure 6 and can be compared.

Because it was found that *Diplococcus pneumonia* died rapidly, not being able to recover it from the toy three hours after contamination, it can be implied that the storage of the toys in an open dry environment will possibly destroy *Diplococcus pneumonia* organisms. However, since *Streptococcus pyogenes* was recoverable until seventy-two hours and *Staphylococcus aureus* was still present on the toy on the fifth day, storage does not appear adequate to destroy these organisms. Since it cannot be known what potential pathogens are present on the toy without culturing, it would not be safe to rely on time alone to destroy organisms.

From the data collected in the hospital it can be seen that toys are harborers of pathogens and potential pathogens can be transferred from a child's throat to his toy. The results of the laboratory study show that organisms do survive on cloth toys, although the survival rate varies for each organism. Thus, the length of the interval between contamination of the toy and handling of it by a second child or person will influence the number of organisms remaining on the toy which could be transferred. No studies of this nature have been reported in literature, thus allowing no comparison
FIGURE 6
MEAN NUMBER OF COLONIES OF STAPHYLOCOCCUS AUREUS, STREPTOCOCCUS PYOGENES, AND DIPLOCOCCUS PNEUMONIA ISOLATED FROM CLOTH STUFFED TOYS, 0 TO 120 HOURS AFTER CONTAMINATION
X ± SE
of the data with similar studies. The next question to be answered is whether organisms on the toy can be transmitted to other patients either by another child using the toy or by being carried to another child by a human carrier.
CHAPTER IV

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

I. SUMMARY

There were two purposes for this research project. The first was to determine if cloth stuffed toys used by children in the pediatric ward of Loma Linda University Hospital could serve as carriers of potentially pathogenic bacteria. It was hypothesized that potentially pathogenic organisms found on the throat culture of a hospitalized child will also be found on the toy the child uses, and conversely, if no pathogens are found in the throat, none will be found on the toy. The second purpose was to determine how long selected bacterial pathogens, Staphylococcus aureus, Streptococcus pyogenes, and Diplococcus pneumonia can survive on the same cloth toys as were used in the selected hospital.

The review of literature showed that studies concerning the hospital environment as a means of disseminating organisms deal with three aspects; a measure of the degree of contamination, determination of transfer of contamination from one source to another, and whether pathogens from a contaminated object or person can cause an infection when transferred to a human. Divergence of opinion regarding fomites as harborers and transmitters of pathogens seems to be related to which of the above aspects was studied. Most studies show, however, that fomites do harbor bacteria. No previous research was found to be done concerning cloth stuffed toys.
More than one method of collecting organisms from fomites was found in literature, but the Rodac culture plate method was felt to be the simplest and most appropriate for this study. Studies regarding the characteristics of the specific organisms demonstrate that they can survive for indefinite periods, no specific time periods being determined.

In the hospital study, a total sample of twenty-four patients with their toys were used. The selected toys were pillow-like in shape and covered with medium weave smooth, printed cotton cloth. The ages of the patients ranged from five months to eleven years. A throat culture was taken of each child on admission. Before giving a toy to the child, control cultures, using the Rodac culture plate, were taken of the front and back of the toy. Cultures were obtained from the front and the back of the cloth stuffed toy after twenty-four, forty-eight, and seventy-two hours of use.

The cultures were incubated at 37° C. for twenty-four to thirty-six hours after which they were macroscopically and microscopically examined. Further specific tests were carried out on those microorganisms suspected of being potential pathogens. Records and gram-stain slides were kept of each type of organism cultured from each patient.

The control cultures showed that no potential pathogens were on the toys when given to the children. Of the twenty-four patients studied, eight had the same pathogens cultured from their toys as were cultured from their throats. Eight of the sample showed no pathogens cultured from their throat or their toys. These two
groups, sixteen out of twenty-four, or 66 percent, agreed with the hypothesis that if potentially pathogenic organisms are cultured from a child's throat they can also be cultured from the cloth stuffed toys he uses in the hospital, and conversely, if no potential pathogens are found in the throat, none will be found on the toy. Because of the nature of the study and the small sample it was not possible to determine the statistical significance. There were four cases in which potential pathogens were found in the throat but not on the toy. In another four cases potential pathogens were isolated from the toy but not from the throat. These eight cases, or 33 percent, did not agree with the hypothesis.

To fulfill the second purpose of the study, three organisms were studied separately in the laboratory. An undiluted growth culture in Difco Todd-Hewitt broth of the selected organisms was systematically sprayed from an aerosol sprayer directly onto the front and back of two toys. Rodac plate cultures were taken from the surface of the toys immediately after spraying, at thirty minutes, one hour, three hours, six hours, twelve hours, twenty-four hours, and every twenty-four hours thereafter until two cultures showed no organisms and ceasing at five days if the organisms were still being cultured from the toys.

Results showed that the *Diplococcus pneumoniae* cannot be cultured from a cloth stuffed toy three hours after spraying. *Streptococcus pyogenes* cannot be cultured from the toy after seventy-two hours, and *Staphylococcus aureus* can still be cultured from the toy five days after spraying.
II. CONCLUSIONS

The findings of this study show that sixteen, or 66 percent, of the cases studied agreed with the hypothesis that potentially pathogenic organisms found on the throat culture of a hospitalized child will also be found on the cloth stuffed toy that the child uses, and conversely, if no pathogens are found in the throat, none will be found on the toy. However, there were eight, or 33 percent, of the cases that disagreed with the hypothesis. These cases that disagree show up an inadequacy in the study. In four of the eight cases that disagreed there were potential pathogens found on the toy but not on the throat. Because these pathogens could have come from the environment they may be cultured from a child's toy even though none are cultured from the child's throat. Thus the data suggests that the hypothesis be modified before further study.

In view of the stated purpose this study reflects only the degree of contamination of cloth stuffed toys showing that they can carry bacterial pathogens and not demonstrating their potential for causing infection in a human host.

From the laboratory portion of the study it can be concluded that the length of survival of organisms on cloth toys depends on the specific organisms present, but that certain organisms may survive five days or longer.

III. RECOMMENDATIONS

Based on the results of this study, these recommendations were made.
1. That cloth stuffed toys should be washable so that all potential pathogens can be removed by washing after use by a child and before giving it to another child.

2. That toys should be confined in the patient's bed in order to prevent other children from using them.

**Recommendations for Further Studies**

1. That a study be conducted to determine the degree of contamination of toys constructed of different types of material such as plastic, vinyl, wood, and synthetic fur.

2. That a study be conducted to ascertain if potential pathogens on a cloth stuffed toy can be transferred to a child using the toy.

3. That a study be conducted incorporating the following improvements:
   
   a. a larger sample of patients.
   
   b. limiting the age of the children used to under three years (based on the results of the age correlation).
   
   c. limiting the handling of toys to a specific child through use of a controlled observational situation.
   
   d. phagotyping of all *Staphylococcus aureus* found and typing of *Diplococcus pneumoniae* and *Streptococcus pyogenes*.
   
   e. cultures taken of more sites on the toy at each culturing time.
   
   f. cultures taken immediately after observing mouth-toy contact.
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APPENDIX
LOMA LINDA UNIVERSITY
Graduate School

CONTAMINATION OF STUFFED CLOTH TOYS
ON A PEDIATRIC UNIT

by
Virginia Lois Fowler
Dynnette Nelson Hart

An Abstract of a Thesis
in Partial Fulfillment of the Requirements
for the Degree Master of Science
in the Field of Nursing

May 1968
ABSTRACT

The purpose of the study was to determine if cloth stuffed toys used by children in the pediatric unit of Loma Linda University Hospital carry microbacterial pathogens that are the same type as those pathogens found in the throat of the child using the toy. A second purpose was to determine how long the selected pathogens, Staphylococcus aureus, Streptococcus pyogenes, and Diplococcus pneumonia, survive on the same cloth toys as used in the selected hospital. Throat cultures were taken of twenty-four patients and each patient was given a cloth stuffed toy selected to be studied. Cultures of the toys were taken at twenty-four, forty-eight, and seventy-two hours after admission using the Rodac culture plate method. After incubation the culture plates were macroscopically and microscopically classified to determine if potential pathogens were present. Of the twenty-four patients studied, eight of the sample had the same potential pathogens cultured from their toys as cultured from their throats, while eight showed no potential pathogens either in their throat or on their toy. These sixteen agreed with the hypothesis that if potential pathogens are cultured from the child's throat, these also can be cultured from the cloth stuffed toy he uses; and also, if no pathogens are found in the throat, none will be found on the toy. There were eight of the twenty-four cases that disagreed with the hypothesis. In four of these cases potential pathogens were found in the throat but not on the toy and the remaining four had potential pathogens on the toy, but none were found in the throat.
Undiluted suspensions of the selected organisms (only one organism each time) were sprayed onto two cloth stuffed toys. The Rodac plate culture was taken from the front and back of each of the toys at determined intervals. Results showed that no *Diplococcus pneumonia* organisms could be isolated from the toy after three hours; no *Streptococcus pyogenes* could be isolated from the toy after seventy-two hours; and *Staphylococcus aureus* was still present on the toy five days after spraying.

Thus, the amount of time intervening between when a toy is contaminated and it is handled by a person will affect the degree and kind of pathogenic organisms transmitted between the two.