



Research Article | Araştırma Makalesi

INVESTIGATION OF PATHOGENIC CONTAMINATION ON TOY MATERIALS THROUGH MICROBIOLOGICAL AND STRUCTURAL CHARACTERIZATION APPROACHES

OYUNCAK MALZEMELERİNDE PATOJENİK KONTAMİNASYONUN MİKROBİYOLOJİK VE YAPISAL KARAKTERİZASYON YAKLAŞIMLARIYLA İNCELENMESİ

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ABSTRACT

Objective: Toys in kindergartens are frequently exposed to environmental contaminants and hand contact, making them potential reservoirs for pathogenic microorganisms. Children, with developing immune systems, are particularly vulnerable to infections. This study aimed to detect *Escherichia coli*, *Staphylococcus aureus*, and coliform bacteria on toys and to characterize toy materials using FTIR, XRD, and SEM.

Methods: Swab samples were collected from toy surfaces under aseptic conditions and transported under cold-chain conditions. Samples were homogenized in buffered peptone water and serially diluted. Coliforms were detected on VRB and BGGLB media, *E. coli* on TBX medium, and *S. aureus* on Baird-Parker Agar with Egg Yolk Tellurite. Identification was confirmed via Gram staining, catalase, DNase, hemolysis, and carbohydrate fermentation tests. FTIR assessed functional groups, XRD examined crystalline structures, and SEM observed surface morphology related to microbial adhesion.

Results: Bacteriological analysis showed *Staphylococcus* spp. (35.33%), *S. aureus* (5%), *E. coli* (1%), and coliforms (3.66%). FTIR revealed diverse polymeric structures, XRD showed varying crystallinity, and SEM indicated heterogeneous surface roughness and irregular microstructures facilitating bacterial adhesion.

Conclusion: Combining microbiological analyses with FTIR, XRD, and SEM provides a comprehensive method for assessing contamination risks. Kindergarten toys can harbor potentially pathogenic microorganisms, and the structural characteristics of toy materials significantly influence microbial persistence.

Keywords: Microbial contamination, toy materials, scanning electron microscopy, Fourier transform infrared spectroscopy, X-ray diffraction

Öz

Amaç: Anaokullarında kullanılan oyuncaklar, çevresel kontaminantlara ve sık el temasıyla mikroorganizmalara maruz kalarak patojenler için potansiyel rezervuar oluşturur. Gelişmekte olan bağışıklık sistemine sahip çocuklar enfeksiyon riski altındadır. Bu çalışmada, seçilmiş anaokullarındaki oyuncaklarda *Escherichia coli*, *Staphylococcus aureus* ve koliform bakterilerin varlığı araştırılmış ve oyuncak malzemeleri FTIR, XRD ve SEM ile karakterize edilmiştir.

Yöntem: Oyuncak yüzeylerinden sürüntü örnekleri steril koşullarda alınmış ve soğuk zincirle laboratuvara taşınmıştır. Örnekler tamponlu peptonlu suya aktarılmış ve seri dilüsyonlarla çoğaltılmıştır. Koliform bakteriler VRB ve BGGLB besiyerlerinde, *E. coli* TBX besiyerinde ve *S. aureus* Baird-Parker Agar (yumurta sarısı tellürit ilaveli) üzerinde tespit edilmiştir. Tanımlama Gram boyama, katalaz, DNaz, hemoliz ve karbonhidrat fermentasyon testleriyle doğrulanmıştır. FTIR fonksiyonel grupları, XRD kristalin yapıyı ve SEM yüzey morfolojisini değerlendirmek için kullanılmıştır.

Bulgular: Bakteriyolojik analizler *Staphylococcus* spp. (%35,33), *S. aureus* (%5), *E. coli* (%1) ve koliformlar (%3,66) oranında bulunmuştur. FTIR çeşitli polimerik yapıları gösterirken, XRD kristalinlikte farklılıklar, SEM ise bakteriyel tutunmayı kolaylaştırabilecek düzensiz yüzey mikro yapıları göstermiştir.

Sonuç: Mikrobiyolojik analizlerin FTIR, XRD ve SEM ile birleştirilmesi kontaminasyon risklerinin kapsamlı değerlendirilmesini sağlar. Anaokulu oyuncakları potansiyel patojenler barındırabilir ve malzeme yapısal özellikleri mikrobiyal kalıcılığı etkiler.

Anahtar Kelimeler: Mikrobiyal kontaminasyon, oyuncak malzemeler, taramalı elektron mikroskobu, Fourier dönüşümlü kızılötesi spektroskopisi, X-ışını kırınımı

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Submitted/Başvuru: 21.12.2025

Accepted/Kabul: 09.03.2026

Published Online/Online Yayın: 08.06.2026



Introduction

Toys constitute an integral component of early childhood development, supporting cognitive, social, and motor skills through play-based learning. In kindergarten settings, these objects are shared frequently among children and are continuously exposed to hands, respiratory droplets, and environmental surfaces.¹ Such intense and repeated contact amplifies the likelihood of microbial deposition and subsequent colonization. Numerous studies have emphasized that children—owing to their developing immune systems, frequent hand-to-mouth behaviors, and prolonged interaction with shared objects—represent a highly vulnerable population to infections transmitted via contaminated fomites. Consequently, toys used in childcare environments have been increasingly recognized as potential reservoirs for pathogenic and opportunistic microorganisms.²

Previous research has documented the presence of a wide range of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and various coliform group members, on surfaces of commonly used toys and educational materials.³ These microorganisms are associated with gastrointestinal diseases, and other clinically relevant conditions, indicating that toy-borne contamination may contribute to infection transmission dynamics within early childhood institutions.⁴ Despite regular cleaning policies recommended for such settings, microbial persistence on toy surfaces often remains underestimated. A critical knowledge gap persists regarding how the physicochemical and structural properties of toy materials influence bacterial adhesion, survival, and biofilm formation potential.

Material composition and surface morphology are known to play significant roles in microbial retention. Factors such as surface roughness, polymeric structure, hydrophobicity, and crystallinity can modulate bacterial attachment strength and long-term persistence. However, comprehensive studies integrating microbiological analyses with material characterization techniques remain limited. Advanced analytical tools such as Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Scanning Electron Microscopy (SEM) offer valuable insights into the chemical functional groups, crystalline phases, and microstructural features that may facilitate or inhibit microbial adherence. Employing these methods in combination provides a holistic understanding of contamination mechanisms on toy surfaces.⁵ SEM analysis provides critical insights into surface morphology and microstructural features that influence microbial adhesion and persistence.

Given the susceptibility of children in kindergarten environments and the potential public health implications of contaminated toys, there is a pressing need to investigate both the microbiological profiles and the structural characteristics of toy materials. This study addresses this need by examining the presence of *E. coli*, *S. aureus*, and coliform bacteria on toys collected from

selected kindergartens and by evaluating the materials' physicochemical properties using FTIR, XRD, and SEM. By integrating these complementary approaches, the research aims to elucidate the interplay between microbial exposure and material composition, thereby contributing to improved risk assessment strategies and informing more effective hygiene and sanitation practices in early childhood settings.

Methods

Three hundred swab samples were collected from six different kindergartens.

Ethical statement

Permission to collect toy samples was obtained from the administrations of the participating kindergartens prior to sample collection. The study did not involve human subjects, personal data, or invasive procedures. Therefore, formal ethical committee approval was not required. All sampling procedures were conducted in accordance with institutional research and ethical guidelines.

Materials

Hydrogen peroxide (H₂O₂), immersion oil, fuksin, mannitol salt Agar (MSA), iodine-lugol solution, Brilliant Green Bile Lactose Broth (BGBLB), Tryptone Bile X-glucuronide Medium'a (TBX), Egg Yolk Tellurite ilaveli Baird Parker Agar (BPA), Violet Red Bile (VRB), swab, and Chemicals and culture media used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated.

Instruments

In this study, in order to observe the band vibrations and tensions of the functional groups in the structure of the toy, BRUKER/VERTEX 70 brand FTIR analysis was performed between 400-4000 cm⁻¹. Similarly, XRD analysis was performed using BRUKER D8 ADVANCE brand device to observe the diffraction of the toy structure against X-rays. SEM analysis was performed with ZEIS EVO LS 10 brand device for the photograph of the morphological appearance of the toy sample.

Preparation of samples

The swab method was used to collect the samples. Swab samples were taken by rubbing the surface for at least 20 seconds under aseptic conditions and were taken to the laboratory under appropriate storage conditions (cold chain).⁶

The study's toy samples included a variety of toys commonly found in kindergarten settings, such as hard plastic toys and soft plush toys. Hard toys were made from synthetic polymers like polyethylene and polypropylene, which had comparatively smoother and less porous surfaces than plush toys, which were mostly made of polyester-based fibrous materials with porous and irregular surface structures. Microbial adhesion and persistence are known to be impacted by these material-

dependent structural variations. Smoother polymeric surfaces typically have a lower capacity for retaining microorganisms, whereas fibrous and porous materials offer greater surface areas and protective niches that promote microbial attachment and retention. Every toy examined was actively used by children in authentic settings

Microbiological analysis

The swabs brought to the laboratory under appropriate storage conditions were transferred to test tubes containing 20 ml of sterile buffered peptone water and then mixed thoroughly with a vortex to ensure that the microorganisms on the cotton (swab) passed into the liquid medium. Then, from this main dilution, appropriate dilutions were made to determine total aerobic mesophilic bacteria, coliform group microorganisms, *Escherichia coli*, and *Staphylococcus aureus*, and incubated in parallel in the relevant media. At the end of the incubation period, the colonies formed were evaluated and identification tests were performed for verification. In line with the results obtained from the laboratory, it was aimed to provide hygiene training to families, persons, and institutions related to children in nurseries and kindergartens.

Detection of Coliform Group Microorganisms

0.1 ml of the prepared dilution was taken and cultured on Violet Red Bile (VRB) medium by pouring method and the medium was incubated for 24 hours at 37°C under aerobic conditions. At the end of this period, cherry-colored colonies forming precipitation with a diameter of 0.3–0.5 mm was evaluated as suspicious coliform group microorganisms. For confirmation purposes, suspicious colonies were transferred to Brilliant Green Bile Lactose Broth (BGBLB) with the help of a loop and then the tubes were incubated at 37 °C for 24 hours. Those forming gas in the tubes after incubation were evaluated as positive.⁷

Detection of *Escherichia coli*

0.1 ml of the prepared master dilution was taken and cultured in Tryptone Bile X-glucuronide Medium (TBX) by pouring method and incubated at 30°C for 4 hours and then at 44°C for 18 hours. After incubation, bluish/green colonies that were positive for indole test were evaluated as *E. coli*.

Detection of *Staphylococcus aureus*

For *S. aureus* count, Baird Parker Agar (BPA) with Egg Yolk Tellurite added was inoculated according to the spread plate method, and the Petri dishes were incubated at 37°C for 24–48 hours. Typical colonies that grew on BPA and were round, 1–3 mm in diameter, shiny, convex, gray, and black (tellurite reaction), with an opaque ring and a transparent zone around it (lecithinase reaction) were evaluated as *S. aureus* suspicious colonies. In order to determine whether these selected colonies caused beta hemolysis, they were inoculated onto blood agar medium and after 24 hours of incubation at 37°C, those

that caused beta hemolysis were selected and other identification tests were carried out.

Gram staining

A loopful of pure culture developed in the medium was taken on the slide. It was spread with physiological saline and left to dry in the air. After the slide was fixed by passing it over the flame 3 times, the preparation was stained with crystal violet and waited 1 minute. Then, it was washed with distilled water and the crystal violet was removed. Iodine-Lugol solution was dropped on the preparation, waited for 1-2 minutes, and then washed with distilled water. Then, it was treated with alcohol for 15 seconds and washed again with distilled water. Finally, the preparation was stained with aqueous fuchsin, waited for 30 seconds, and washed again with distilled water. After the preparation was dried in the air, immersion oil was dropped and examined under a 100-magnitude objective. As a result of the Gram stain, purple-colored, cluster-like colonies consisting of cocci were accepted as positive.⁸

Deoxyribonuclease (DNase) test

A single colony was taken from a 24-hour pure culture spot-plated onto DNase medium and incubated for 18–24 hours at 37°C. At the end of the incubation period, 1N HCl acid was dropped onto the colony that grew and the petri dishes were examined on a dark background. Openings on the colony were evaluated as positive.⁹

Catalase test

A 24-hour culture was transferred to a clean slide using a loop and mixed with a drop of 3% hydrogen peroxide (H₂O₂). The formation of air bubbles as a result of the release of oxygen within seconds was evaluated as a positive reaction.

Aerobic fermentation of carbohydrates test

After cultivation on Purple Agar Base medium, it was left for 24–48 hours of incubation at 37°C. Isolates that changed the purple color to yellow at the end of the incubation period were evaluated as positive.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 20.0; IBM Corporation, Armonk, NY, USA). Descriptive statistics were used to summarize the prevalence and frequency of microbial contamination detected on toy surfaces. The distribution of bacterial isolates was expressed as frequencies and percentages. Graphical representations were used to illustrate the distribution of microbial contamination among the analyzed samples.

Results

Descriptive statistical analysis was conducted to evaluate microbial contamination on toy surfaces. The frequency of contamination was determined by calculating

percentages based on the total number of analyzed toy samples. Since the primary aim of the study was to describe the distribution and frequency of contamination, no inferential statistical tests were applied.

SEM

Figure 1 shows scanning electron microscopy (SEM) images of a soft, furry toy. SEM allows us to examine the surface structure of materials by obtaining high-resolution images. In Figure 1a, the structures on the surface of the toy are clearly visible. On the surface, spherical structures with a diameter of about 2 micrometers and fine lines around them are observed. The magnification of the image is 25,000X, the working distance is 12.5 mm and the signal type is SEM. In Figure 1b, a larger area of the toy's surface was examined. The surface shows intertwined fine fibers and gaps between them. The magnification of the image is 1,000X, the working distance is 12.5 mm, and the signal type is again SE1. The spherical structures and fine fibers in the images reflect the microscopic features that make up the soft, fuzzy texture of the toy. SEM analysis was used to describe the surface morphology of toy materials, which is crucial for microbial adhesion and persistence, rather than to assess antibacterial activity. Bacterial attachment and retention are directly impacted by surface characteristics like fiber density, roughness, and microstructural irregularities. Beyond traditional microbiological enumeration, SEM allows for the mechanistic interpretation of microbial contamination through the high-resolution visualization of these structural features. As a result, SEM analysis is a valuable supplementary method for comprehending the connection between microbial persistence and surface structure.

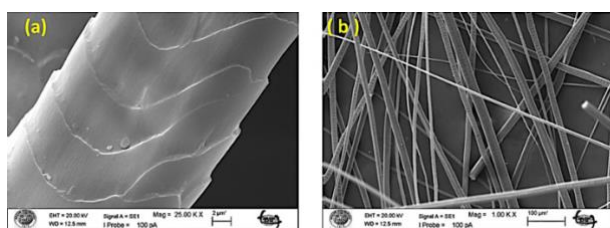


Figure 1. SEM characterization image of (a) the furry toy for clear visible and (b) the furry toy for general visible.

FTIR

A FTIR analysis of the furry toy is given in figure 2. The spectrum was measured in the wavelength range of 500 cm^{-1} to 4000 cm^{-1} . The broad peak around 3300 cm^{-1} indicates the presence of hydroxyl (-OH) groups.^{10,11}

The peaks around 2900 cm^{-1} indicate the presence of aliphatic C-H bonds. The peak around 1700 cm^{-1} indicates the presence of carbonyl (C=O) groups. The peak around 1600 cm^{-1} indicates the presence of aromatic rings.¹² These results suggest that the materials used in the production of the toy may be synthetic polymers such as polyurethane, polyethylene, or polyester.

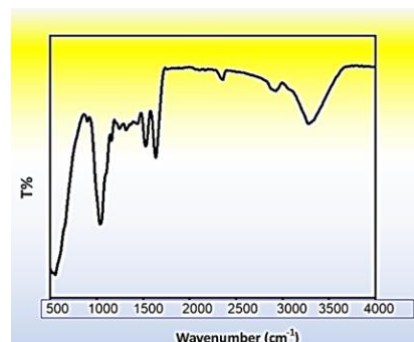


Figure 2. FTIR analysis of the furry toy.

XRD

The report shows the results of X-ray diffraction (XRD) analysis of a furry and soft toy in Figure 3. XRD is an analysis technique that provides detailed information about the crystal structure and phases of materials. The spectrum was measured in the range of 20° degrees to 90° degrees 2θ angle. A high intensity and sharp peak in the 20°-30° degree range indicates the presence of a crystal structure. Lower intensity and broader peaks in the 30°-50° degree range indicate amorphous components of the crystal structure. Lower intensity and scattered peaks in the 50°-90° degree range represent other components of the crystal structure.

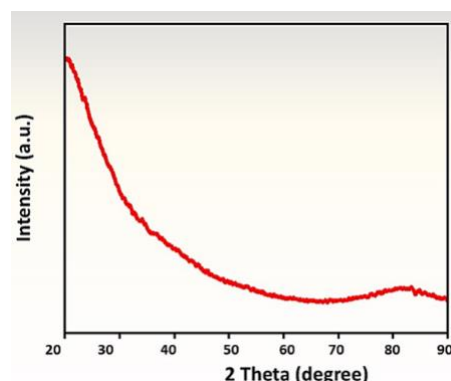


Figure 3. XRD analysis of a furry toy.

Bacterial studies

Determination of *Escherichia coli*, *Staphylococcus spp.* and *Staphylococcus aureus* were given in Figure 4. Using the pouring technique, 0.1 ml of the generated master dilution was cultured in Tryptone Bile X-glucuronide Medium (TBX) and incubated for 4 hours at 30°C and 18 hours at 44°C. According to Figure 4a colonies that were bluish/green and had a positive indole test after incubation were determined to be *E. Coli*. After the incubation process was completed, colonies exhibiting bluish-greenish color tones were observed on selective and differential media. As a result of the biochemical analyses applied to these colonies, confirmation was made with a positive indole test and it was definitely determined that these colonies belonged to the *Escherichia coli* species. These findings support the typical phenotypic characteristics of *E. coli* and its identifiability with specific biochemical methods such as the indole test. Petri dishes containing *Staphylococcus*

species and *Staphylococcus aureus* bacteria were incubated for 24 to 48 hours at 37°C (Figure 4b). *S. aureus* suspicious colonies were identified as typical colonies developed in BPA, which were spherical, 1–3 mm in diameter, glossy, convex, gray, and black in color (tellurite reaction), with an opaque ring and a clear zone surrounding it (lecithinase reaction).

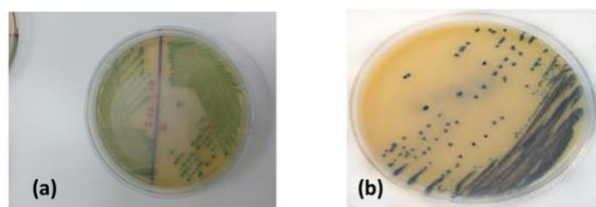


Figure 4. Determination of (a) *Escherichia coli* (b) *Staphylococcus* spp. vs *Staphylococcus aureus*.

Petri dishes containing *Staphylococcus* species and *Staphylococcus aureus* bacteria were incubated for 24 to 48 hours at 37°C (Figure 5). *S. aureus* suspicious colonies were determined to be typical colonies that develop in BPA, are spherical, 1–3 mm in size, glossy, convex, gray, and black in color (tellurite ratios), and have an opaque ring and a clear zone surrounding it (lecithinase samples). The provided images show the results of the DNase test (Figure 5a), the Mannitol test (Figure 5b), and the Aerobic fermentation test (Figure 5c), respectively. According to Figure 5a, the DNase test showed that microorganisms exhibited the ability to produce DNase enzymes. A positive result indicates that the microorganism produces DNase enzymes and can degrade DNA. Figure 5b shows that microorganisms have the ability to ferment mannitol sugar. In Figure 5c, a positive test result indicates that the microorganism can grow in the presence of oxygen.

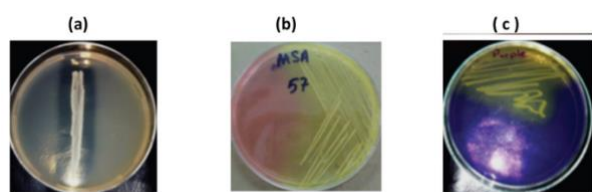


Figure 5. Bacterial identification tests: (a) DNase test, (b) Mannitol fermentation test, and (c) Aerobic fermentation test.

The analysis of 300 swab samples taken from 6 kindergartens detected 106 (35.33%) isolates of *Staphylococcus* spp., 15 (5%) isolates of *S. aureus*, 3 (1%) isolates of *E. coli*, and 11 (3.66%) isolates of the Coliform group (Figure 6). While the percentage of bacterial species detected in toys is given in Figure 6a, the number of toy samples collected for analysis is shown in Figure 6b. Detection of many infectious agents such as *Staphylococcus*, *E.coli*, Coliform in toys shows that they endanger children's health. It is essential to keep toys clean to reduce the spread of diseases. This study also supports previous reports. Table 1 compares this study

with previous studies. According to Table 1, the bacterial rates detected in this study support previous reports.

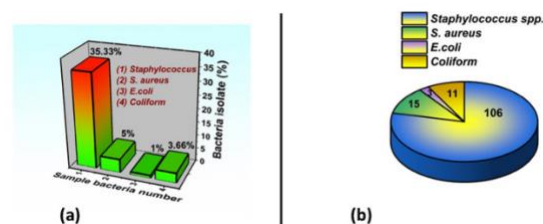


Figure 6. (a) The histogram of bacteria types ratio (b) The graphic of total bacteria types.

Table 1. The comparison of previous studies and this study using kindergartens.

No	Type and ratio of bacteria	Ref
1	<i>Sphingomonas paucimobilis</i> (>603 CFU/cm ²) <i>Panotea</i> spp. (>575 CFU/cm ²) <i>Alloiococcus otitidis</i> (>569 CFU/cm ²) <i>Rothia dentocariosa</i> (>567 CFU/cm ²) <i>Kocuria rosea</i> (>538 CFU/cm ²)	13
2	<i>Staphylococcus aureus</i> (2.5 × 10 ⁴ CFU hands ⁻¹) <i>Klebsiella pneumoniae</i> (1 × 10 ⁴ CFU hands ⁻¹)	14
3	Coliform (13.5% - 20%)	15
4	<i>Staphylococcus</i> spp. (35.33%) <i>Staphylococcus aureus</i> (5%) <i>E. coli</i> (1%) Coliform (3.66%)	This study

Discussion

This study demonstrated that toys used in kindergartens can serve as reservoirs for various pathogenic microorganisms, including *Staphylococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, and coliform bacteria, posing a significant risk for cross-contamination among children. The prevalence rates detected in this study—35.33% for *Staphylococcus* spp., 5% for *S. aureus*, 1% for *E. coli*, and 3.66% for coliforms—are consistent with previous reports on microbial contamination of children's toys, confirming that such objects play a critical role in pathogen transmission within communal environments.^{2,13,14} Children, due to frequent hand-to-mouth contact, immature immune responses, and shared interaction with objects, are particularly vulnerable to infections originating from contaminated surfaces. Even low prevalence of enteric bacteria such as *E. coli* signals potential fecal contamination, which can have critical implications for gastrointestinal infections in early childhood environments.^{2,13,14} This study integrates microbiological findings with SEM, FTIR, and XRD analyses to provide a complementary perspective on bacterial persistence on toy surfaces used in kindergarten settings.

SEM analysis revealed that the furry toy possesses a complex surface morphology composed of intertwined microfibers and approximately 2 μm spherical particles, providing an ideal structure for microbial adhesion and colonization (Figure 1). These observations corroborate earlier studies indicating that surface topography and

roughness significantly increase bacterial retention by protecting cells from mechanical removal during cleaning procedures.¹⁵ These analyses also provide insight into material selection and manufacturing-related surface features that may influence microbial persistence. FTIR analysis further indicated that the toy is composed of synthetic polymers such as polyurethane, polyethylene, or polyester, which—despite their durability—may create favorable conditions for microbial persistence on surfaces.¹⁰⁻¹² XRD analysis revealed a mixed crystalline–amorphous structure, suggesting that the material’s physical characteristics may influence its interaction with microbial cells and its overall susceptibility to contamination. For instance, smoother, less porous surfaces are generally less conducive to long-term microbial survival, whereas rougher and more chemically complex polymers can harbor persistent microbial communities. The combined use of surface morphology, chemical composition, and structural analyses provides a mechanistic framework rather than a purely descriptive assessment of contamination. The microbial retention capacity of toys is significantly influenced by their type and material composition. The highly porous and fibrous surface morphologies of plush toys made of polyester fibers offer protective microenvironments for microbial adhesion and persistence. On the other hand, compared to fibrous materials, the surfaces of hard plastic toys made of polymers like polyethylene and polypropylene are generally smoother and less porous, which lowers microbial retention. By influencing bacterial attachment strength, survival, and resistance to mechanical removal during routine handling and cleaning, these material-dependent variations have a direct impact on contamination dynamics.

These findings underscore the importance of both toy material composition and surface morphology in microbial survival, highlighting the necessity of strict and regular cleaning procedures in settings where young children frequently share objects. This study is limited by its cross-sectional design and the lack of quantitative adhesion or biofilm assays; however, it provides a strong basis for future mechanistic and intervention-focused studies.

In conclusion, this study demonstrates that kindergarten toys can harbor potentially harmful microorganisms and that the structural and chemical characteristics of toy materials play a critical role in microbial adherence and persistence. The combined application of microbiological methods with FTIR, XRD, and SEM constitutes a comprehensive framework for understanding contamination dynamics and informs practical interventions to enhance child safety in educational environments.

Limitations

It is important to recognize the various limitations of this study. First, temporal fluctuations in contamination levels are not taken into consideration by the cross-sectional design, which represents microbial contamination at a single time point. Second, the study

excluded viral and fungal pathogens, which may also influence contamination dynamics, in favor of concentrating on specific bacterial indicators. Third, despite the description of various toy materials, microbial retention capacity was not directly measured using quantitative adhesion assays or biofilm formation experiments. Lastly, there was no systematic evaluation of environmental factors like humidity, cleaning frequency, and handling intensity. The knowledge of contamination mechanisms would be further strengthened by future research that includes quantitative surface–microbe interaction analyses, longitudinal sampling, and wider pathogen screening.

Compliance with Ethical Standards

Permission to collect toy samples was obtained from the administrations of the participating kindergartens prior to sample collection. The study did not involve human subjects, personal data, or invasive procedures. Therefore, formal ethical committee approval was not required. All sampling procedures were conducted in accordance with institutional research and ethical guidelines.

Conflict of Interest

The author declared no conflict of interest

Author Contributions

GI; analysis, collected datas and methodology, EEA; edited datas and writing, EA; writing and checking, OA; supporting and collected datas, HA; collected datas and methodology.

Financial Disclosure

None.

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