

**BACTERIAL ISOLATES, PRESENT ON SURFACE OF TOYS IN CHILD CARE CENTERS, IN AL-RASS CITY, AL-QASSIM REIGON. K.S.A.****Dr. Sarah Ali<sup>\*1</sup>, Mashael M. Al-Harbi<sup>2</sup> and Dr. Syed R. Rahman<sup>3</sup>**<sup>1</sup>Assistant Professor, Microbiology. Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, Al Qassim, Saudi Arabia.<sup>2</sup>Department of Medical Laboratories, College of Applied Medical Sciences, Qassim University, Buraydah, Al Qassim, Saudi Arabia.<sup>3</sup>Medical Director, Al Shigah PHC, AlShigah. Buraydah. AlQassim. Saudi Arabia.**\*Corresponding Author: Dr. Sarah Ali**

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**ABSTRACT**

**Introduction:** Toys have always been an important part of childhood and play an important role in child's physical and mental development. In children care center the toys are available for a large number of children and toys are passed from child to child and become contaminated through handling or by children putting their mouth to them. With increasing numbers of women in the workforce, day care centers have been increasing in number and the children at this age are at a higher risk of gastroenteritis diseases. In order to provide awareness for care centers department, we aimed to test toys sample to detect if they contained bacteria or not. **Methods:** This was an observational study using the cross-sectional study design, to determine species of pathogenic bacteria on toys samples to provide awareness in children, staff who work in care centers and also for families, whose children were registered with the care centers. The study included 25 samples from 2 kindergarten and 1 child care center. The samples were tested at Qassim University Department Of Applied Medical Sciences Microbiology Laboratory. Sample were taken by, sterile swab from the surface of each toy, and inoculated in different bacteria media e.g. blood agar, macConkey and chocolate agar. and incubated for 24 - 48 hr at 37°C. Bacterial species Differentiation was done by gram stain, biochemical tests, API for Staphylococci and API 20E. **Results:** Bacteria isolated from 25 samples, included, Staphylococcus. Aureus (40%) from 10samples, Staphylococcus CoN (60%) from 15 samples, Klebsiella pneumoniae (4%) from 1sample, Bacillus spp (72%) from 18 sample, Alpha hemolytic Streptococcus (4%) from 1sample, and Micrococci (4%) from 1sample. 16 samples had more than one bacterial profile when cultured. **Conclusion:** The result of this study confirmed, that the toys serve as media for transmission of diseases. kindergarten and child care center must improve follow, standard hygiene procedures for protecting the children, infants, and workers in the facilities.

**KEYWORDS:** Kindergarten, Child care center, Toys, Bacteria.**1. INTRODUCTION**

With increasing numbers of women in the workforce, day care centers have been increasing in number and the children at this age are at a higher risk of gastroenteritis diseases. Children in child care facilities are generally much younger than those in schools, sometimes as young as a few weeks old.

The behaviors of very young children (eg, crawling, hand to mouth activity) increase their exposure to contaminants (fungi and bacteria) in dust, on surfaces or in toys and other objects. There are four primary ways to spread illness among children airborne/respiratory, fecal-oral, blood-body fluid and direct contact.

In health care settings, the environment has been found to be naturally laden with microorganisms, and in child

care centers, diaper changing and incomplete toilet training could promote bacterial growth".<sup>[4]</sup> The care Centre is a place where infants and children are kept for a particular period of time.<sup>[3]</sup>

Child-care facilities appear to provide a setting with many opportunities for exposure and transmission of bacteria and viruses.<sup>[5]</sup> Small children have habits that facilitate the dissemination of diseases, such as putting their hands and objects in their mouths, very close interpersonal contact, fecal incontinence during the phase prior to the acquisition of sphincter muscle control, the absence of the habit of hand washing and other hygienic practices and the need for constant direct physical contact with adults.<sup>[6]</sup>

Children in daycare nurseries have more infections than children cared for elsewhere. mainly because of direct transmission between children, contact or respiratory droplet transmission and inadequate hand hygiene. Although indirect transmission of infection via the nursery environment has not been studied extensively, it is likely to play a role. Previous research on bacteria in the nursery environment has shown positive cultures in 10-60% of samples, depending on location, but almost all bacteria isolated were of low pathogenicity. Children, especially children aged three years and under, have a high frequency of infectious disease episodes.<sup>[7]</sup>

One of the major health challenges in modern life of children aged 0-6 years is the use of day-care centers. Numerous studies throughout the world have described an increased risk of infectious diseases associated with attending daycare centers.<sup>[9]</sup> Infections in health care settings can be considered within the framework of endemic infections, outbreaks, and antimicrobial resistance. Respiratory tract infections, urinary tract infections, skin and soft tissue infections are the most frequent endemic infections.

Outbreaks are common, with influenza A virus and gastrointestinal infections which caused by *Escherichia coli* O157:H7, *Salmonella* species and *shigella* are the most frequent and severe.<sup>[10]</sup> Child care environment facilitate the spread of enteric infections because of diapering, confined spaces, and children's unhygienic habits.<sup>[11]</sup>

Bacterial enteric infections have a huge impact on human health, particularly among the pediatric population.<sup>[12]</sup> Transmission of enteropathogens is increased not only among children who attend child care, but also among their family members and child-care workers. In prospective study of diarrhea in 20 child-care centers, (10 percent) of 331 family contacts of children with diarrhea developed diarrhea.<sup>[13]</sup>

### General objective

To determine species of bacteria present on toys of children in child care center (pre-school) in AL-Rass city.

### Secondary objective

1. To collect toys samples from children care center and kindergarten in AL-Rass city by randomly selected location.
2. To determine pathogenic bacterial species in all samples
3. To provide awareness about safety of children's and infants in care centers.

## 3. METHODOLOGY

### 3.1 Overview of the Study Design

This was an observational cross-sectional study. It was done to determine species of pathogenic bacteria on toys samples to provide awareness in children, staff who work

in care centers and also for families, whose children were registered with the care centers. The study included collection of several toys, from AL-Rass city. Sterile bags were used to collect and transport them to Qassim university microbiology laboratory. Sampling was done by sterile cotton swab moistened with sterile normal saline, multiple samples were taken from all toys, samples from multiple locations within and on the surface of each toy, using separate swab, and immediately inoculated in culture medias [Blood agar, MacConkey and Chocolate agar]. Gram smear stain from the swabs and Culture plates, was performed for Differentiation and analytical profile index Microbial system e.g API 20E, API for *Staphylococcus*, biochemical tests, Oxidase, Catalase, Coagulase & IMViC, were performed to confirm bacterial isolates. The Analytical Profile Index (API) is a miniaturized panel of biochemical tests compiled for identification of groups of bacteria .API test is required different time for incubation dependent which type of API are used.

The Possible exposure in this study are storage place, type of toys (hard toys or soft toys), an environment of place, different categories of toys according to age, contact period, water contact and humidity, and the outcome is species of pathogenic bacteria on toys samples. The study conducted during the period of January to May 2016 in AL-Rass city.

### 3.2 Study Sample

Toys samples collected from care centers in AL-Rass, al-Qassim during the period from January to May2016 in AL-Rass city 2016.

The samples size included 25 toys samples (N=25) from child care centers (N= 9), and from kindergarten" pre-school" (N=16). Toys collected from different locations (north, south and east) in AL-Rass City.

### Inclusion criteria

Toys from child care centers, kindergarten and nursery schools available in different parts of AL-Rass city.

### Exclusion criteria

Toys from homes and other cities.

### 3.3 Data Collection

Data collected from the randomly tested samples in AL-Rass. Toys samples collected in sterile bag and labeled with ID number, date, time, collector name and location. Samples analysis conducted in Al- Qassim university laboratory by using analytical profile index (API 20E), oxidase for gram negative and biochemical test "catalase and coagulase". Analytical profile index is commercial miniaturized biochemical test panels that cover a significant number of clinically-important groups of bacteria and also give accurate identification based on extensive databases and are standardized easy-to-use test systems. The kits include strips consist of micro tubes (cupules) and contain up to 20 miniature biochemical

tests. All are quick, safe and easy to perform before collection of the samples wear the gloves to prevent the contamination.

Sterile cotton swab was used to take multiple samples, within and on the surface of each toy. Samples were cultured, using different bacteria media e.g. blood agar, macConkey, and chocolate agar. Inoculated swab in bacterial media and, plates were incubated for 24 - 48 hr at 37°C. Smears from Swabs and bacterial cultures were made and gram stained to identify gram positive or gram negative bacteria, s.

#### Gram-negative bacteria

By sterile wire take 3-4 colonies of unknown bacteria from media, mixed into 5 ml of sterile saline to make suspension of bacteria, and inoculated into API 20E analytical profile index to identify.

Enterobacteriaceae API 20 E. API 20 E strip labelled with specimen number and data and distribute 5ml of sterile distilled water into bottom of tray to provide a moist atmosphere which prevent drying of the strip. Holding the strip at a slight angle up from the table top to inoculate the bacterial suspension into each well with sterile pipette to eliminate any bubbles forming in the wells CIT, VP AND GEL, test wells were completely filled. Sterile mineral oil added to some reaction e.g. LDC, ODC, ADH, H<sub>2</sub>S and URE (which had line under their name in strip) to provide anaerobic condition. Incubation of the strip overnight 18-24hr at 37°C. After incubation the reactions were recorded, test reagents were added to some compartments, metabolism of the organism produced color change, and test results were tabulated.

The tests on the strip, plus oxidase test were used to determine the first seven digits of the profile number. This is usually sufficient to determine the identity

organism by using the profile number in code book, or by using API web software.

#### For gram-positive bacteria

Biochemical test "catalase & coagulase" were performed. The catalase test is used primarily in differentiation between certain genera and species of bacteria. Catalase is an enzyme present in most cytochrome containing aerobic and facultative anaerobic bacteria. The test is performed by Using a loop, pick an area of growth from an 18-24 hr old pure colony. Place the growth on a clean microscope slide. then, using a Pasteur pipette or a dropper, place a drop of 3% H<sub>2</sub>O<sub>2</sub> over the organism on the slide. Observe for immediate bubbling. [+ve reaction]. Identification of streptococci by haemolysis they produced on blood agar, followed by differentiating biochemical tests to Identify them. Pathogenic Staph species (e.g. S. aureus) was confirmed using the coagulase test and mannitol salt agar. There are 2 kinds of coagulase enzyme [bound and free]. in tube agglutination test, were performed.

#### 3.4 Data Analysis Plan

The data entered in Microsoft excel software, and analyzed using descriptive statistics with frequency and percent and the results will be presented using tables, pie charts and graphs.

#### Ethical Considerations

Ethical approval for the study obtained from the department review committee. Informed consent obtained from administrators of child care centers, kindergarten, and departments which have nursery school for their participation in this study.

#### Study Limitations

Some of the limitations were number of samples and short time of study, effect of time period (from collect the sample until processing), cleaning toys before sampling.

## 4.0. RESULTS

Table. 1: Bacteria isolated per total number of samples (N=25).

Bacterial species	Number of sample showing the mentioned bacterial species.	Percentage per 25 Samples
Staphylococcus. Aureus	10	40%
Staphylococcus CoN	15	60%
Klebsiella pneumoniae	1	4%
Bacillus spp	18	72%
Alpha Hemolytic Streptococcus	1	4%
Micrococci	1	4%

Bacteria isolated from 25 samples, included, Staphylococcus. Aureus (40%) from 10 samples, Staphylococcus CoN (60%) from 15 samples, Klebsiella pneumoniae (4%) from 1 sample, Bacillus spp (72%)

from 18 samples, Alpha hemolytic Streptococcus sp (4%) from 1 sample, and Micrococci (4%) from 1 sample. 16 samples had more than one bacterial profile when cultured.

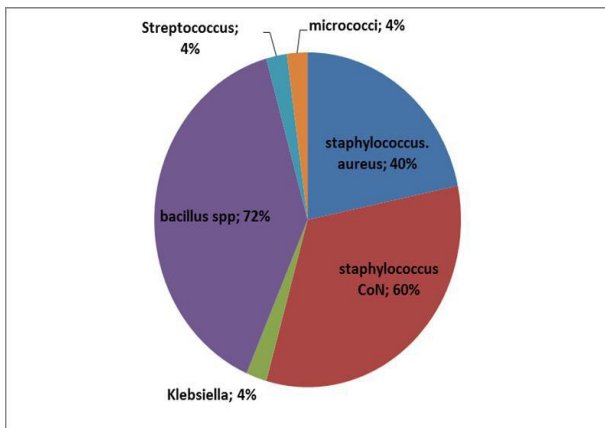


Figure. 1: Bacteria isolated per total number of samples (25).

From 25 samples, Bacteria isolated from 14 soft samples and 11 hard samples. Percentage of Staphylococcus aureus isolated in soft samples was more than that isolated from hard samples. (50% vs 27.2%). Percentage of Staphylococcus CoN isolated in hard samples was more than that isolated from soft samples (63.6% vs 57.1%). Percentage of Bacillus spp isolated in hard samples was more than that isolated from soft samples (81.8% vs 64.28%). Alpha hemolytic Streptococcus was isolated only in hard samples (9%). Klebsiella pneumoniae and Micrococci was isolated only in soft samples (7.14%, 7.14%).

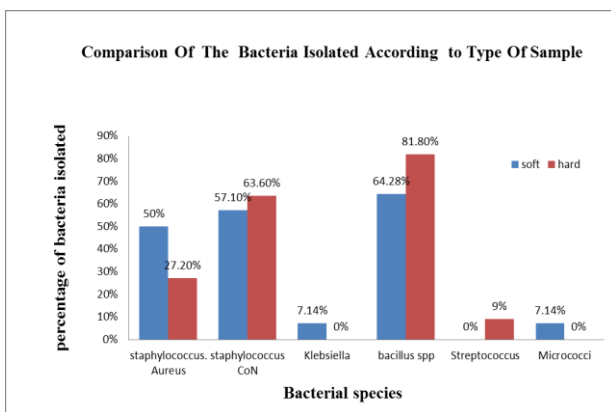


Figure 2: Comparison of the bacteria isolated according to type of Toy sample.

Table No. 2: Comparison of Bacterial isolates with type of Location.

Place	No of sample	Bacterial species					
		staphylococcus CoN	Staphylococcus Aureus	bacillus spp	Alpha hemolytic Streptococcus	Klebsiella	Micrococci
kindergarten 1 (6-7 years)	6	50%	50%	50%	0%	0%	0%
kindergarten2 (6-7 years)	10	60%	20%	90%	10%	0%	10%
child care center (3months – 5years)	9	66.6%	55.5%	66.6%	0%	11%	0%

In kindergarten 1 Bacteria isolated from 6 samples, showed the following; Staphylococcus CoN (50%), Staphylococcus. Aureus (50%), and Bacillus spp (50%), In kindergarten 2, Bacteria isolated from 10 samples, gave the following; Staphylococcus CoN (60%), Staphylococcus. Aureus (20%), Bacillus spp (90%), Alpha hemolytic Streptococcus (10%), and Micrococci (10%). In child care center, Bacteria isolated from 9 samples, showed the following: Staphylococcus CoN (66.6%), Staphylococcus. Aureus (55.5%), Bacillus spp (66.6%) and Klebsiella pneumoniae (11%).

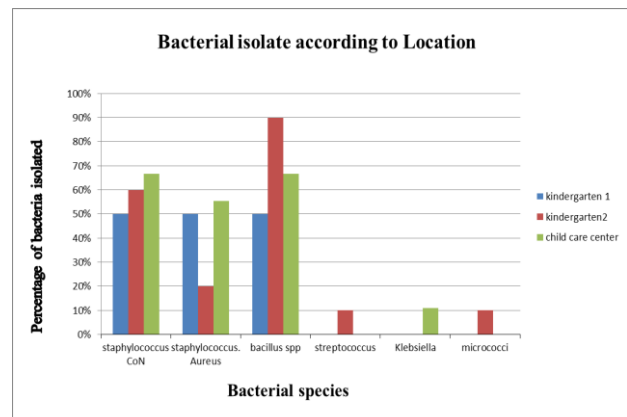


Figure. 3: Bacterial isolate according to type of Location.

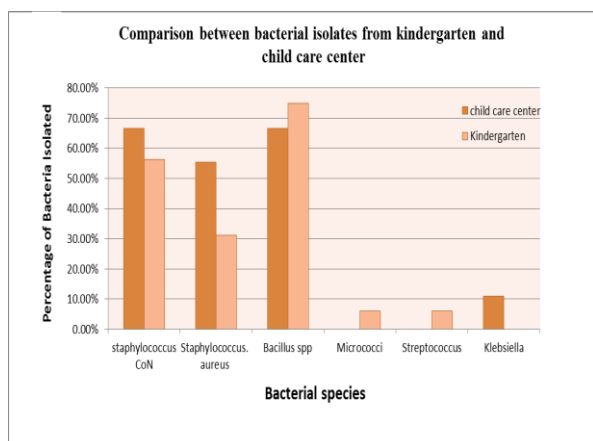


Figure. 4: Comparison between bacterial isolates from kindergarten and child care center.

Bacterial isolates of *staphylococcus CoN* was more in child care center than kindergarten (66.6% vs 56.25%), and the same for *Staphylococcus aureus*, more in child care center than kindergarten (55.5% vs 31.25%). *Micrococci* and *Alpha hemolytic Streptococcus* was found to be more in kindergarten than child care center (6% vs 0%). Bacterial isolates of *Klebsiella pneumoniae* more in child care center than kindergarten (11% vs 0%).

Table 3: Bacterial isolate per type of samples and location.

Location	Total no of sample	No	Type of sample	staphylococcus CoN	staphylococcus Aureus	bacillus spp	Klebsiella pneumoniae	Alpha hemolytic Streptococcus	micrococci
Kindergarten1	6	2	Hard	50%	0%	50%	0%	0%	0%
		4	Soft	50%	75%	50%	0%	0%	0%
child care center	9	3	Hard	100%	66.6%	100%	0%	0%	0%
		6	Soft	50%	50%	50%	16.6%	0%	0%
Kindergar-ten2	10	6	Hard	50%	16.6%	83.3%	0%	16.6%	0%
		4	soft	70%	25%	100%	0%	0%	25%

## 5.0. DISCUSSION

The purpose of this study was to find bacterial contamination in toys in child care centers and kindergarten. We sampled surface contamination on toys using sterile cotton swabs in four daycare classrooms. Bacteria were isolated on macConkey and blood agar plates. Culturing methods identified viable bacteria on all toys sampled in the study. *Bacillus* spp were the most commonly cultured bacteria, followed by *Staphylococcus* spp.<sup>[5]</sup> Similar results found in our study bacteria isolated from 25 samples was more for *Bacillus* (72%), followed by *Staphylococcus* spp [*Staphylococcus aureus* (40%) and *Staphylococcus CoN* (60%)].

Bacteria isolated from toys and play areas in restaurants and shopping malls in Al-Riyadh, Saudi Arabia revealed bacterial contamination with *Streptococcus* (5%) and *Klebsiella pneumoniae* (5%).<sup>[2]</sup> also in our study show the same percentage of bacteria isolated for *Streptococcus* (4%) and *Klebsiella pneumoniae* (4%). Other studies conducted in toys in waiting room in children's hospitals. that is more possibility to contamination than toys in child care centers.

In our study, we compare between soft and hard toys (figure2). We Found Percentage of *Staphylococcus aureus* isolated in soft samples was more than that isolated from hard samples (50% vs 27.2%). Percentage of *Staphylococcus CoN* and *Bacillus* spp isolated in hard samples was more than that isolated from soft samples (63.6% vs 57.1%), (81.8% vs 64.28%) respectively.

While *Alpha hemolytic Streptococcus* was isolated only in hard samples (9%). *Klebsiella pneumoniae* and *Micrococci* was isolated only in soft samples (7.14%, 7.14%).

Comparison Bacterial Profile With age of children in child care centers and kindergarten. (Figure 10) didn't show significant result [*Staphylococcus CoN*, *Staphylococcus aureus*, and *Bacillus* spp] where approximately in all place. But *Streptococcus Alpha hemolytic* was found only in Kindergarten that means the age from 6-7 years similarly, same result found for *Micrococci*. While *Klebsiella* absent in higher age group, found it in child care centers [3months-5years].

## 6.0. CONCLUSION

The result of this study confirms that the toys serve as media for transmission the disease. The microorganisms a pose risk for immunocompromised children. These facilities must improve the suitable hygiene procedures for protecting the children, infant, and workers in the facilities. some recommendation have been provided for this facilities about the role of toys in disease transmission among children in child care centers and correct method for wash the toys.

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