American Journal of Infection Control 47 (2019) 456–458

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Brief Report

Are ball pits located in physical therapy clinical settings a source of pathogenic microorganisms?

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Key Words:
Ball pits
Pediatric physical therapy
Microbial colonization
Opportunistic pathogenic bacteria
Opportunistic pathogenic yeasts

Clinical, therapeutic ball pits commonly used by physical therapists to provide sensory stimulation to children were investigated for microbial colonization. Due to the permissive and hospitable environment provided by these ball pits, microorganisms can accumulate to levels that increase the ease of transmission to exposed individuals. Our study found considerable microbial colonization in ball pits located in clinical settings, including 8 opportunistic pathogenic bacteria and 1 opportunistic pathogenic yeast.

The popularity of ball pits has increased in the general population since commercial restaurant chains installed them nationwide in the 1980s. Ball pits are often contaminated with visible dirt, vomit, feces, or urine providing an origin and permissive environmental factors for microbial contamination. Numerous types of bacteria have been identified in ball pits located in community settings, including normal human skin bacteria, as well as opportunistic pathogens such as *Staphylococcus aureus* and various enteric bacteria. Besides human-associated microorganisms, some ball pits have been found to contain zoonotic-associated organisms that have also been identified as causing serious infections in humans, including *Pasteurella multocida*.[1]

Ball pits are also commonly used in pediatric physical therapy to help provide stimulation to children with sensory and motor impairments. Currently, identification of national standards, or protocols, for cleaning these enclosures and their contents remains elusive. Accordingly, clinics may go days or even weeks between cleanings, which may allow time for microorganisms to accumulate and grow to levels capable of transmission and infection. This risk increases if the individual has skin lesions or abrasions, providing a portal of entry for immunocompromised individuals in general.

To ascertain the level of microbial colonization or presence in therapeutic ball pits at any given time, regardless of cleaning procedures, we conducted a study on 6 clinical ball pits located in community clinics. The goal of this study was to determine if there was a difference in the amount of bacteria found between each clinic and to identify the microorganisms found in the ball pits.

METHODS

Sample sites included in this study were 6 ball pits located in inpatient physical therapy clinics or outpatient clinics in the state of Georgia. Nine to 15 balls were randomly selected from different depths of each sampled ball pit at the respective clinic. Samples were collected by swabbing the entire surface of each ball using BD flocked swabs from the ESwab Collection and Transport System (model 220245) (Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were inoculated on tryptic soy agar plates and grown for 24 hours at 33°C. Following incubation, plates were examined and the number of colony-forming units (CFUs) was recorded.

To identify the bacteria, cultures were plated on Biolog BUG agar plates (Thermo Fisher Scientific, Inc, Waltham, MA) with 5% sheep's blood and incubated for 24 hours at 33°C. Following incubation, these cultures were used to inoculate the respective Biolog inoculation fluid and 100 μL of the solution was added to each well in a 96-well Biolog biochemical assay plate. The plate was incubated at 33°C and read in a BioTek Synergy H1 Multi-Mode microplate reader (BioTek

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Funding/support: Funding was provided by a University of North Georgia Presidential Innovation Award (to A. Robinson and D.B. in 2016), the University of North Georgia Department of Physical Therapy, and the University of North Georgia Department of Biology.
Conflicts of interest: None to report.

https://doi.org/10.1016/j.ajic.2018.09.031
0196-6553/

Table 1
Number of microorganisms represented as CFU/ball found in each physical therapy clinic

	Clinic A CFU/ball	Clinic B CFU/ball	Clinic C CFU/ball	Clinic D CFU/ball	Clinic E CFU/ball	Clinic F CFU/ball
Mean	24,707.2	170,818.2	1,464.545	914.2857	2,665.714	211.4286
SD	76,703.5	227,587.7	1,445.796	1,084.064	1,782.394	267.4216
Median	95.5	30,000	1,180	420	2830	110
Minimum	6	30,000	10	50	300	30
Maximum	243,000	712,000	3,000	2,830	5,560	740

CFU, colony-forming unit; *SD*, standard deviation.

Instruments, Inc, Winooski, VT) at 545 nm every 2 hours for 24 hours. The Biolog GEN III system (Thermo Fisher Scientific, Inc) was then used to identify the bacteria based on the results of the biochemical assays read by the plate reader.

Yeast were isolated on Sabouraud dextrose agar and grown for 24 hours at 30°C. These overnight plate cultures were inoculated into Remel RapID Inoculation Fluid (Thermo Fisher Scientific, Inc) and then added to the RapID panel. The panels were then incubated at 30°C for 4 hours and subsequently scored. Final identification was made by the Remel ERIC software suite (Thermo Fisher Scientific, Inc).

RESULTS AND DISCUSSION

We found considerable variability among samples in the number of recoverable CFUs between clinics and locations in the ball pits (Table 1). Of the 6 clinics, Clinic B had the most balls (93%), with $>3.0 \times 10^4$ CFUs, whereas Clinic A had the fewest. In contrast, 36% of the balls from Clinic A had $<3.0 \times 10^1$ CFUs (Table 1). The considerable variation in the recoverable CFU suggests that clinics utilize different protocols for cleaning and maintenance of their ball pits. Bacterial colonization was found to be as high as thousands of cells per ball, which clearly demonstrates an increased potential for transmission of these organisms to patients and the possibility of infection in these exposed individuals.

Further, among the microorganisms isolated from the ball pits, we identified 31 bacterial species and 1 species of yeast (Table 2). Nine of the identified microorganisms were opportunistic pathogens (Table 2). Among human-associated bacteria, *Enterococcus faecalis* can cause endocarditis, septicemia, urinary tract infections, and meningitis.[2] *Staphylococcus hominis* can cause bloodstream infections, and it was reported as a cause of sepsis in a neonatal intensive care unit.[3] *Streptococcus oralis* is known to cause endocarditis, adult respiratory distress syndrome, and streptococcal shock.[4] *Acinetobacter lwofii* was reported to cause septicemia, pneumonia, meningitis, urinary tract, and skin infections.[5]

There are also a number of cases in which the primary environmental-associated microbes identified in this study have been indicated in human infections. *Raoultella terrigena* is typically found in soil or aquatic environments, but it has been reported to be associated with endocarditis and sepsis in post-surgical patients.[6] *Psychrobacter immobilis* is also typically found in fish, meat, and poultry, but it has been reported to be the cause of a nosocomial ocular infection in a newborn.[7] *Klebsiella variicola*, historically isolated from plants, can cause bloodstream infections.[8] *Mycobacterium aichiense/novocastrens* was reported as the cause of a wound infection on a child's hand.[9]

Rhodotorula mucilaginosa, a yeast that was identified from the clinical ball pit samples, has a high affinity for plastics, which lead to colonization on many types of medical equipment, including central venous catheters that result in multiple in cases of fungemia in immunocompromised individuals.[10]

Table 2
All bacterial species identified and organized based on gram-positive Firmicutes, gram-positive Actinobacteria, gram-negative Proteobacteria, H, E, and their pathogenicity

	Origin
Gram-positive microorganisms	
Bacillus fastidiosus	E
Bacillus galactosidilyticus	E
Bacillus mojavensis/subtilis	E
Bacillus plakortidis	E
Bacillus sporothermodurans	E
Bacillus thuringiensis/cereus	E
Bacillus lentus	E
Bacillus horikoshii	E
Sporolactobacillus terrae	E
Enterococcus faeclis[*,†,‡,§]	H
Macrococcus brunensis	E
Paenibacillus xylanilyticus	E
Staphylococcus hominis ss hominis[†,‡,‖]	E
Streptococcus oralis[‡]	E/H
Streptococcus sobrinus	H
Aerococcus viridans	E/H
Vagococcus salmoninarum (26C)	E
Gram-positive Actinobacteria	**Origin**
Micrococcus flavus[§]	E
Mycobacterium aichiense/novocastrense	H
Gram-negative Proteobacteria	**Origin**
Acinetobacter lwoffii[‡]	H
Klebsiella variicola[#]	E/H
Moraxella caprae	E
Pseudoxanthomonas yeogluenesis	E
Pseudomonas agarici	E
Pseudomonas fragi	E
Pseudomonas pertucinogena	E
Psychrobacter immobilis[**]	E/H
Raoultella terrigena[‡,††]	E/H
Stenotrophomonas rhizophila	E
Yeast	**Origin**
Rhodotorula mucilaginosa[‡‡]	E/H

Note. Bold font indicates different names phylogenic groups of microorganisms.
E, environmental-associated microbe; *H*, human-associated microbe.
*Urinary tract infections.
†Bacteremia.
‡Endocarditis.
§Wound infections.
‖Septicemia.
#Bloodstream infections.
**Conjunctivitis.
††Sepsis.
‡‡Fungaemia.

CONCLUSIONS

A considerable amount of microbial colonization was found in pit balls located in pediatric physical therapy clinical settings. In addition, 9 species of opportunistic pathogens were identified among the isolated microorganisms. Although it is normal to see human microbes wherever humans are present, further study of the amount of colonization should be performed and, if warranted, standardized

cleaning protocols developed to limit the presence of opportunistic pathogens in this environment.

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