Gambling With Your Health: Bacterial Contamination on Casino Gaming Chips

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Abstract **The casino environment, consisting of employees and customers, can present a risk for exposure to infectious diseases, especially bacterial diseases that are found on casino gaming chips. The purpose of this study was to replicate a study from 2011 to determine bacterial microorganisms on casino chips. A total of 26 chips (13 used actively in a casino and 13 never used from a chip manufacturer) were used for the study. Bacteria and fungi development were found in statistically significant numbers ($p < .05$). Contamination was found on used versus unused chips based on the location being tested, namely the obverse (the side of the chip bearing the head or principal design), reverse, or edge of the chip—with overall results being statistically significant for the presence of pathogenic contaminants. This study also determined that the chips showed the presence of *E. coli* at statistically significant levels.**

Introduction

According to Saldmann (2008), the list of illness-causing bacteria and viruses that can be spread through casual hand-to-hand or inanimate object-to-hand contact includes: *E. coli, Tatumella ptyseos, Serratia plymuthica, Citrobacter freundii, Proteus penneri, Erwinia,* and *Helicobacter pylori*. By coming into contact with objects that have been contaminated by individuals who are carriers of these illness-causing bacteria and viruses, these infectious diseases can be spread through casual human contact. Further, if bacteria or viruses are deposited on an object, (e.g., someone infected with human influenza sneezes without covering their mouth), then the infectious bacterial organisms can live from several hours to up to 5 months on inanimate objects, depending on the environmental conditions (Brady, Fraser, Dunlop, Paterson-Brown, & Gibb, 2007; Kramer, Schwebke, & Kampf, 2006; Rutala, White, Gergen, & Weber, 2006; Saldmann, 2008).

In the medical and healthcare field, hand washing practices are determined by monitoring the bacterial levels located on objects such as keyboards and wireless communication devices (Brady et al., 2007; Rutala et al., 2006). The results of these studies show that despite continual use and cleaning, disinfectants were continually required to ensure that disease-causing microorganisms were controlled to safe levels (Brady et al., 2007; Rutala et al., 2006). This vigilant approach is critical, especially in light of research that disease-causing viruses can remain on everyday surfaces such as door knobs, desk tops, and chairs—even after disinfectants have been used to sanitize the contaminated area (Terpstra et al., 2007).

One of the major barriers to effectively controlling the spread of infectious diseases is proper personal hygiene, particularly hand washing. The Centers for Disease Control and Prevention (CDC) has worked to create the Clean Hands Count (CHC) campaign in an effort to "create and support coordinated, sustained initiatives to significantly improve health and save lives through clean hands" (Centers for Disease Control and Prevention [CDC], 2018a). Research has shown that public restrooms are a source of bacterial and viral infection because of improper hand washing (Allwood, Jenkins, Paulus, Johnson, & Hedberg, 2004; Bakalar, 2005; Berry, Mitteer, & Fournier, 2014; de Kort & Velthuijsen, 2011; Guinan, McGuckin-Guinan, & Sevareid, 1997; Oldfield, 2017). Further, if people are using public restrooms in a casino, then cross-contamination can occur on casino gaming chips, because studies have shown that on average, 35% of the U.S. population does not wash their hands after using the restroom (Altekruse, Yang, Timbo, & Angulo, 1999; Berry et al., 2014; Byrd-Bredbenner et al., 2007; "Did you wash your hands," 1996; Filion, Kukanich, Chapman, Hardigree, & Powell, 2011; Guinan et al., 1997).

It should be noted that even though 65% of the U.S. population has been found to

wash their hands after using the restroom, the duration of hand washing does not reach the recommended time to ensure that hands are effectively cleaned. Berry and coauthors (2014) found that the average time that individuals wash hands after using the restroom was 8.1 s, with the range being 0.52–57.7 s. The Food and Drug Administration recommends that when washing hands, you should:

> "(3) Rub together vigorously for at least 10 to 15 seconds while: (a) Paying particular attention to removing soil from underneath the fingernails during the cleaning procedure, and (b) Creating friction on the surfaces of the hands and arms or surrogate prosthetic devices for hands and arms, finger tips, and areas between the fingers" (U.S. Department of Health and Human Services, 2013, pp. 46–47).

Alternatively, CDC recommends that when washing hands, you should "rub your hands together vigorously for at least 15 seconds, covering all surfaces of the hands and fingers" (CDC, 2018b).

Casino employees and customers can be at risk for exposure to infectious diseases, especially bacterial diseases, through the handling of chips. A study by Mc Keown and coauthors (2011) was designed to determine if infectious bacteria were present on chips that were used by casino workers and customers by comparing the bacteria counts of these chips to new, never-used-before chips.

The purpose of this replication study was to determine to what degree the results obtained from the Mc Keown and coauthors' 2011 study, where both bacteria and fungi were present in statistically significant numbers on both the unused (factory) and used (in use at casinos) chips, were due to happenstance or instead indicate a serious health issue. The secondary purpose of this study was to determine if *E. coli* or coliforms are among the illness-causing bacteria found on the chips being studied. The information gathered from this study will provide recommendations that can reduce and prevent infectious bacterial disease among casino workers and customers.

Methods

The protocol for this study closely follows that which was outlined in Mc Keown and coauthors (2011) with changes made to the protocol outlined below. This study employs a case-control design to determine if infectious bacteria exist on chips. The in-use chips were purchased at a table game in the amount of $100 in $5 chips, resulting in a total of 20 chips being purchased for the study. The $5 denomination was chosen as a chip that is available at the various table games and is actively in use in games with minimum bets ranging from $1–$25.

Then, 13 chips that have never been used in a casino were compared with 13 chips that had been in play at an undisclosed casino in the Midwest. It was determined that the number 13 was used in the original study because the primary investigator was self-funding the study and that was how many blood-agar Petri dishes could be purchased.

In this study, a total of 20 chips ($5 denomination each) were collected from four different casinos, with 4 chips from a casino in the Gulf Coast and the other 15 chips (5 each) from three different casinos in Las Vegas, Nevada. Chips were randomly chosen in equal numbers from the four casinos until 13 chips had been tested. Each chip contains three sides (obverse or front, reverse or back, and side or rim), so a total of $n = 78$ tests were performed: 39 for the used chips, and 39 for the control group (never-used chips).

Obverse and reverse sides of the chips were determined based on the chip design and positioning of colored stripes in relation to wording and casino label. Chip labels closely oriented with the wording on the edge of the chip were considered the obverse side of the chip. In the Mc Keown and coauthors' 2011 study, chips were randomly removed from sterilized plastic containers marked as either used or unused using sterilized forceps.

In this study, two biologists performed the tests and directly removed the chips from the zip-sealed plastic bags that they were collected in from the casinos. One biologist performed the tests on the used chips and a different biologist performed the tests on the new, unused chips. The biologists wore neoprene gloves while handling the chips for testing. Between the testing of each chip, the testing area and gloves were sterilized with an alcohol solution of 70% ethanol. Each chip was then swabbed for bacteria using 6-in. sterile cotton-tipped applicators that had been dipped into a sterile solution of elution fluid containing 1% tween and 0.3% lecithin (Gaonkar, Geraldo, Shintre, & Modak, 2006).

The obverse side of the chip surface area was swabbed first, followed by the reverse side, and finally the rim. To gauge the degree in which the process might generate unique findings, we reversed swabs 22–27 to determine if swabbing order affected the results of the study. Additionally, we introduced a different bottle of sterile elution fluid at swab number 49. Both bottles of sterile elution fluid were made at the same time and both sterile elution fluids were tested before and after the study was completed. These steps were taken to determine that the elution fluids were not contaminated.

Swabs were then directly streaked across numbered blood agar Petri dishes, with the number corresponding to the location of the chip being swabbed to determine reactionary issues based on microorganism growth. For this study, larger Petri dishes were inadvertently acquired, so lines were drawn to create three equal areas. Each area was labeled either with an O, R, or E to reference the obverse (front), reverse (back), or edge (side) of the chip. The Petri dishes were also labeled with an identifier indicating from which of the four casinos it originated.

Once all the Petri dishes had been swabbed, they were placed upside down (optimal growing condition) in a growth incubator set at 37 °C for 48 hr. After 48 hr, the Petri dishes were removed from the incubator and placed in a refrigerated cooling area until the results were analyzed. This protocol for growing bacteria from contaminated surfaces is standard procedure (Bykowski & Stevenson, 2008). At the end of the study, the purchased chips were used in other studies, then returned to the respective casinos and redeemed for the cash value.

Results

We used analysis of variance (ANOVA) to measure the bacterial growth comparisons between the control and casino-used chips. We used the statistical program Stata version 10.1, which is considered a powerful statistical analysis package, to perform these tests. A probability of $p < .05$ was used for determining significant differences between the control versus casino-used chips for bacterial growth. A total of 78 samples (39 from each set of control chips and casino-used chips)

offered enough statistical power (for $\alpha = .05$, $SD = 0.50$, $N = 78$; power = 0.865) to determine the statistical significance noted above.

First, the plates were examined to determine the results (Figure 1). We used microscopic examination to identify cellular morphology and reaction (Figure 2). The bacteria cultured from the unused (control) chips were morphologically similar throughout each plate (Table 1). Bacteria on the unused casino chips consisted of gram-positive bacillus (rodlike) populations on all plates analyzed (Table 2). According to the World Health Organization, *Corynebacteria*, *Propionibacteria*, and *Staphylococcus epidermidis* are common gram-positive bacteria that colonize human hands. Although gram-positive bacteria colonize the hands to a greater extent than gram-negative bacteria, a greater diversity of bacteria, fungi, and viruses are key features in the human hand microbiome compared to alternative sources of bacterial populations on inanimate objects (Cosseau et al., 2016; Wenzler, Fraidenburg, Scardina, & Danziger, 2016). Although outside the scope of this experimental design, the population of bacteria found on the unused chips might originate from the manufacturing and packaging process rather than direct human contact, thus explaining the low diversity of bacteria present on the surface of the chips.

The blood agar plates containing bacteria from the used chips displayed higher diversity of bacteria and fungi (Table 2). There were roughly 32% fungi and 68% bacteria on each plate. With the use of selective *E. coli* media and coliform media, we detected the presence of *E. coli*, a type of coliform and common food poisoning-related bacterium (Addis & Sisay, 2015). Plates 2, 4, 5, 6, 8, 11, and 12 contained both gram-positive and gram-negative bacillus and gram-negative cocci (spherical-like) bacteria. Furthermore, the identification of gram-negative cocci bacteria on plate 11 suggests the presence of genera *Neisseria*, *Moraxella*, or *Kingella*, which are causative agents for meningitis, sinusitis, and bronchopneumonia, and can be transmitted by genital-to-hand contamination (Wenzler et al., 2016; Zapka et al., 2011).

The presence of capsular and lipopolysaccharides increases pathogenicity and antiphagocytic qualities suitable for evading the human immune system and can provide genetic diversity for increased multidrug-resistant populations (Arora, Devi, Chadha, & Malhotra, 2009). The differences in bacteria and morphology found is typical of fomites that have been in contact with a multitude of people.

FIGURE **1**

Sample Blood Agar Plate

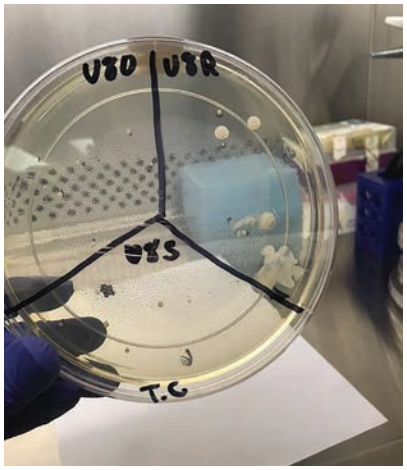
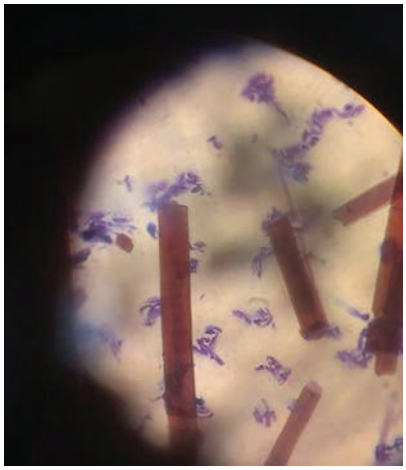


FIGURE **2**

Microscopic Examination



Limitations of the study include genus and species identification of the diverse microbial communities present on used and unused chips using molecular identification, such as DNA sequencing, genomics, or proteomics. Additionally, swabbing might underestimate the total populations on the various surfaces of the chips, because swabbing does not access microbes embedded in the textured layers of the surface. The human hand influences the spread of disease, leaving and picking up microbes with each touch. With the use of standardized methods and increasingly larger studies, we will increase our understanding of the impact of casino chip sanitation on health outcomes.

Of the 78 tests completed, each test produced results that are considered usable for this study. We counted the number of bacteria or fungi colonies that grew in the agar Petri dish. For bacteria, the 78 usable results had a mean of 14.03 colonies and a standard deviation of 7.61 with a range of 1–33 colonies; alternatively, the fungi resulted in a mean of 1.44 colonies and a standard deviation of 1.92 with a range of 0–10 colonies. The *E. coli* test showed a mean of 2.1 colonies and a standard deviation of 3.74 with a range of 0–19 colonies. The coliform test was negative for each case.

The ANOVA results [$F(1,76) = 43.56$, $p < .001$] indicated a statistically significant difference between the amount of bacteria found on used versus unused chips. According to the Bonferroni results, used chips have a higher mean score related to the number of bacteria found than that of unused chips, with a significance of $p < .01$. This study's measure of explained variation, however, shows that 36.43% of the variance in bacteria levels is explained by the differences between used and unused chips. Additionally, the fungi results were also statistically significant [$F(1,77) = 99.89$, $p < .001$], where 56.79% of the variance is explained by the difference between the used and unused chips. Finally, the *E. coli* results were also statistically significant [$F(1,77) = 92.22$, $p < .001$], where 54.82% of the variance is explained by the difference between the used and unused chips.

ANOVA was also performed to determine any differences in the swabbed areas (i.e., obverse, reverse, and edge). The bacteria, fungi, and *E. coli* found were not statistically significant for bacteria [$F(2,77) = 1.19$, $p > .05$], fungi [$F(2,77) = 0.68$, $p > .05$], or *E. coli* [$F(2,77) = 1.87$, $p > .05$]. The variance between the differences in the sections was 3.07% for bacteria, 1.77% for fungi, and 4.74% for *E. coli*.

Finally, the bacteria, fungi, and *E. coli* found on the obverse, reverse, and edge

TABLE **1**

Control (Unused) Casino Gaming Chip Results

Chip #	Surface	Total # of Colonies	Size	Shape	Color	Margin	Elevation	Total # of *E. coli*	Total # of Coliforms	Gram Stain (+ or -)	Bacteria Morphology	Isolated Colonies (RNA Later)	# of Fungi
1	Obverse	11	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	33						0	0	+	Bacillus	1	0
	Edge	9						0	0	N/A	N/A	N/A	0
2	Obverse	15	SM	Round	Yellow, gray	Smooth	Raised	0	0	+	Bacillus	1	0
	Reverse	15						0	0	N/A	N/A	N/A	0
	Edge	16						0	0	N/A	N/A	N/A	0
3	Obverse	27	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	24						0	0	N/A	N/A	N/A	0
	Edge	11						0	0	N/A	N/A	N/A	0
4	Obverse	12	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	12						0	0	N/A	N/A	N/A	0
	Edge	10						0	0	N/A	N/A	N/A	0
5	Obverse	17	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	14						0	0	N/A	N/A	N/A	0
	Edge	24						0	0	N/A	N/A	N/A	0
6	Obverse	19	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	17						0	0	N/A	N/A	N/A	0
	Edge	29						0	0	N/A	N/A	N/A	0
7	Obverse	19	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	14						0	0	N/A	N/A	N/A	0
	Edge	23						0	0	N/A	N/A	N/A	0
8	Obverse	27	SM	Round	Yellow, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	22						0	0	N/A	N/A	N/A	0
	Edge	22						0	0	N/A	N/A	N/A	0
9	Obverse	23	SM	Round	White, gray	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	8						0	0	N/A	N/A	N/A	0
	Edge	17						0	0	N/A	N/A	N/A	0
10	Obverse	20	SM	Round	White, yellow	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	11						0	0	N/A	N/A	N/A	0
	Edge	19						0	0	N/A	N/A	N/A	0
11	Obverse	22	MD, SM	Round	White	Smooth	Raised	0	0	+	Bacillus	1	0
	Reverse	20						0	0	+	Bacillus	1	0
	Edge	17						0	0	N/A	N/A	N/A	0
12	Obverse	11	SM	Round	White	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	25						0	0	N/A	N/A	N/A	0
	Edge	10						0	0	N/A	N/A	N/A	0
13	Obverse	33	SM	Round	White	Smooth	Raised	0	0	N/A	N/A	N/A	0
	Reverse	29						0	0	N/A	N/A	N/A	0
	Edge	18						0	0	N/A	N/A	N/A	0

Note. We performed gram stain, bacterial morphology, isolated colonies, and fungi tests only on chips/petri dishes/colonies that were different. A lot of the colonies throughout the plates looked identical so we would isolate one of the colonies as a representation of the group. We isolated at least one colony out of all the colonies of the same group.
SM = small; MD = medium; N/A: not applicable.

TABLE **2**

In-Use (Used) Casino Gaming Chip Results

Chip #	Surface	Total # of Colonies	Size	Shape	Color	Margin	Elevation	Total # of *E. coli*	Total # of Coliforms	Gram Stain (+ or -)	Bacteria Morphology	Isolated Colonies (RNA Later)	# of Fungi
1	Obverse	5	SM	Round	Yellow, gray	Smooth	Raised	15	0	N/A	N/A	N/A	0
	Reverse	10						5	0	N/A	N/A	N/A	3
	Edge	8						11	0	N/A	N/A	N/A	1
2	Obverse	20	LG, MD, SM	Round	White, yellow, gray	Smooth, rigid	Raised	14	0	+	Bacillus	1	5
	Reverse	8						6	0	N/A	N/A	N/A	1
	Edge	11						5	0	N/A	N/A	N/A	0
3	Obverse	19	MD, SM	Round	White, yellow	Smooth	Raised	4	0	N/A	N/A	N/A	4
	Reverse	27						1	0	N/A	N/A	N/A	4
	Edge	10						2	0	N/A	N/A	N/A	2
4	Obverse	16	LG, MD, SM	Round, rhizoid, filamentous	White	Smooth, rigid	Raised, flat	4	0	N/A	N/A	N/A	3
	Reverse	9						2	0	–	Bacillus	1	3
	Edge	9						6	0	N/A	N/A	N/A	2
5	Obverse	6	MD, SM	Round	Yellow, gray	Smooth	Raised	4	0	N/A	N/A	N/A	3
	Reverse	12						2	0	–	Bacillus	1	5
	Edge	9						5	0	N/A	N/A	N/A	4
6	Obverse	12	LG, MD, SM	Round	White, yellow, gray	Smooth, rigid	Raised	4	0	+	Bacillus	1	3
	Reverse	10						8	0	N/A	N/A	N/A	4
	Edge	10						2	0	N/A	N/A	N/A	4
7	Obverse	21	MD	Round	White, gray	Smooth	Raised	0	0	N/A	N/A	N/A	10
	Reverse	12						2	0	N/A	N/A	N/A	6
	Edge	8						3	0	N/A	N/A	N/A	3
8	Obverse	8	LG, MD, SM	Round, rhizoid	White, yellow, gray	Smooth, rigid	Raised, flat	19	0	N/A	N/A	N/A	2
	Reverse	8						2	0	N/A	N/A	N/A	4
	Edge	6						3	0	–	Bacillus	1	3
9	Obverse	11	MD, SM	Round	Yellow, gray	Smooth	Raised	7	0	N/A	N/A	N/A	3
	Reverse	10						3	0	N/A	N/A	N/A	2
	Edge	8						2	0	N/A	N/A	N/A	2
10	Obverse	5	MD, SM	Round	Yellow, gray	Smooth	Raised	1	0	N/A	N/A	N/A	1
	Reverse	2						1	0	N/A	N/A	N/A	2
	Edge	1						2	0	N/A	N/A	N/A	1
11	Obverse	12	MD, SM	Round	White, yellow, gray	Smooth	Raised	11	0	–	Cocci	1	4
	Reverse	9						0	0	N/A	N/A	N/A	3
	Edge	5						0	0	N/A	N/A	N/A	2
12	Obverse	12	LG, MD	Round, rhizoid	Yellow, gray	Smooth, rigid	Raised, flat	1	0	N/A	N/A	N/A	3
	Reverse	7						1	0	+	Bacillus	2	3
	Edge	7						1	0	+	Cocci	1	3
13	Obverse	1	MD, SM	Round	White, yellow, gray	Smooth	Raised	1	0	N/A	N/A	N/A	1
	Reverse	2						1	0	N/A	N/A	N/A	2
	Edge	3						3	0	N/A	N/A	N/A	1

Note. We performed gram stain, bacterial morphology, isolated colonies, and fungi tests only on chips/petri dishes/colonies that were different. A lot of the colonies throughout the plates looked identical so we would isolate one of the colonies as a representation of the group. We isolated at least one colony out of all the colonies of the same group.
SM = small; MD = medium; LG = large; N/A = not applicable.

($p < .001$) of the chips were statistically significant; however, the amount of explained variation for each test was low at 8.12%, 7.66%, and 6.95% for bacteria; 1.90%, 2.36%, and 1.38% for fungi; and 8.41%, 9.61%, and 6.24% for *E. coli*, respectively.

Discussion

As illustrated above, both bacteria and fungi were found in statistically significant amounts on used and unused chips. This finding aligns with the Mc Keown and coauthors (2011) study, which found:

> "Further microscopic examination of the cell arrangements of the yellow colonies, found on plates 1, 4, 24, 28, 36, 43, 46, 49, 53, 56, 68, 71, and 77, were diplococcic and in tetrads, which means that this was most likely a hand bacterium known as *Micrococcus luteus* (Greenblatt et al., 2004). The fungus showed conclusively under a microscope to be a fungus; however, without expensive DNA sequencing it was not possible to determine which type. Moreover, the fungus resulted in complete hemolysis (rupture or destruction of red blood cells) within the agar Petri dish, also known as beta-hemolysis (β-hemolysis). This increased hemolysis suggested that the fungi were capable of being pathogenic."

With the increased awareness of disease-causing microorganisms and the previous pandemics associated with influenza, these results show that chips can be carriers of organisms that can cause illness in susceptible populations (e.g., older people who tend to spend time at casinos, or infants/toddlers who find colorful chips laying around a hotel room or cruise ship stateroom).

An undercover investigation by *The Today Show* found just as many germs on the handle of a slot machine (373, well above the failure mark of 100) as on elevator buttons (370) (Rossen & Davis, 2015). The cleanliness of casino hotels and cruise ships are constantly being monitored by their respective health districts; unfortunately, the Vessel Sanitation Program 2011 Operations Manual created by CDC has no specific information regarding cleaning and sanitizing of the casino area. Every other area within a cruise ship is listed, with specific requirements and sanitation protocols—except for the casino (CDC, 2011; Cramer, Blanton, & Otto, 2008). Even the Southern Nevada Health District, which monitors hotels and casinos in the Las Vegas area, has only four items in a casino that are required to be cleaned and sanitized in an effort to control and prevent norovirus: "Casino cage counters, gaming chair backs, contact areas of gaming tables, and table game cup holders" (Southern Nevada Health District, 2007).

While this study was conducted using chips from four casinos compared with one casino in the study by Mc Keown and coauthors (2011), it only explored one specific denomination, specifically, the $5 chip. Currently, there are hundreds of casinos around the world where chips are used and chips are available in multiple denominations, ranging from $1–$500 or higher; however, the $5 chips are actively used in just about all casinos and are available in large quantities.

Conclusion

After testing for multiple types of pathogens on multiple chips from multiple casinos, tests are being conducted to determine the best method for cleaning and sanitizing chips to ensure a healthy population, or if the chips should be redesigned to control for the ability to harbor these microorganisms. For example, we placed a chip in liquid bleach for 24 hours with no noticeable discoloration, in addition to placing a chip in an autoclave with no noticeable effects to the gaming chip. While these are two basic methods of sterilization, tests are being conducted on methods of sanitation that would be practical and usable within the casino industry. The eventual goal is to determine effective disease-prevention strategies for the safe handling and use of chips based on the presence of significant levels of infectious bacteria.

As a result, this study shows that additional studies need to be performed, and are being performed, to determine precisely the amounts and types of microorganisms that can be found on chips. Due to limited funds, the variability of chip denominations and casinos was sacrificed. In addition, limited funds dictated the amount of testing that was performed. Continued studies on casino chips will include DNA profiling of the microorganisms in addition to testing for possible viral pathogens.

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