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## **Original Research Article**

# Pathogenic Microbial Contamination on Cakes from Some Major Bakers in Yenagoa, Bayelsa State, Nigeria

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Abstract: Cake is a sweet and tasty baked product consumed globally and can be a source of food poisoning or microbial food contamination. The aim of this study is to isolate and identify pathogenic microorganisms in cakes. Ten (10) cake samples obtained from major bakers in Yenagoa metropolis, Bayelsa state, Nigeria were analyzed, and the Isolation and identification of microorganisms was done using standard microbiological procedures. A total of ninety (90) isolates were isolated in this study on Day 1, Day 3 and Day 7 based on microscopic and biochemical characteristics. The percentage occurrence of micro-organisms isolated on Day1, Day3 and Day7 respectively were Aeromonas Spp (3.1%, 0% and 34%); Bacillus Spp (6.3%, 3.5% and 0%); Candida albicans (21.9%, 27.6% and 27.6%); Escherichia coli (31.2%, 17.2% and 13.8%); Klebsiella spp (0%, 3.5% and 0%); Micrococcus spp (0%, 0% and 10.3%); Proteus spp (6.3%, 6.9% and 0%); Pseudomonas spp (9.4%, 6.9% and 0%); Salmonella spp (0%,10.3% and 20.7%); Staphylococcus aureus (9.4%,10.3% and 6.9%); Staphylococcus epidermidis (3.1%, 6.9% and 17.2%) and Streptococcus spp (9.4%, 6.9% and 0%). E. coli was the highest occurring microbe from cake samples in Day 1 (31.2%), and second highest in Day 3 (17.2%); while Candida albicans was the second highest in Day 1 (21.9%), highest in Day 3 (27.6%) and highest in Day 7 (27.6%). The findings from this study depicts that cakes used in parties and events can be contaminated by several microorganisms and this can serve as a source of food contamination and poisoning. However, bakers should be deliberate regarding proper personal hygiene, storage containers/conditions and vending equipment to avoid the contamination of cakes by microorganisms.

Keywords: Food contamination, Cakes, Pathogenic microrganisms.

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## **INTRODUCTION**

Cakes are baked and are consumed on a daily basis by a lot of persons that most of the time use cold and hot drinks alongside [1]. The main ingredient in cakes includes milk, eggs, sugar and flour. Other ingredients are flavors, fats, baking powder, yeast, nuts, fruits and colors [2].

Microbial spoilage of cake is the *process in* which the quality of cakes deteriorates to the extent that it renders the cake unacceptable for human consumption. Microbial spoilage is the major problem that causes deterioration in bakery products, and it's mainly caused by moulds, yeasts and occasionally by bacteria [3]. The interference between low pH, high and low water activity (aW), and filling materials may support the growth and production of toxins by microorganisms even when the individual ingredients do not support growth. Cakes and bakery products filled with custard, cream, and sauces can be spoiled by microorganisms because of the ingredients that are added after baking, such as icing, nuts, toppings, and cream. Most products, because of low water activity allow only molds to grow. However, many fillings support the growth of spoilage bacteria, especially if they have high water activity, near to neutral pH, and contain high protein ingredients such as meat, egg or milk. Ambient temperatures, pH levels between 5.4-7.5, and water activity in range of 0.75-0.98 promotes spoilage of baked products with mold, yeast, and spore forming bacteria. And cakes have water activity (aw) levels above 0.94 which is an important factor that influences spoilage of the products [4]. The pathogenic bacteria that have been isolated and identified on cake samples were *Bacillus spp*, *Pseudomonas spp*, Salmonella spp, Escherichia coli, Staphylococcus spp,

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Micrococcus spp, Aeromonas spp, Shigella spp, Vibrio spp, Micrococcus spp, Aspergillus and Penicillium [5].

## **MATERIALS AND METHODS**

#### Sample Collection and Processing

Ten freshly baked cakes from Ten (10) different cake vendors in Yenagoa metropolis were aseptically handled and promptly transported to the laboratory for analysis. The cake samples were soaked in sterile water for 30 minutes to dissolve. Each sample was subjected to serial dilution and inoculated in duplicate onto MacConkey agar, CLED agar, Salmonella Shigella agar and Saboraud agar plates. Exactly 0.1ml from the tube labeled  $10^{-6}$  was inoculated on to the agar plates, followed by spreading with the aid of a sterile bent glass rod. The plates were incubated at  $37^{\circ}$ C for 24hours. The total colonies were calculated in colony forming units per ml (CFU/ml) (Ayanda *et al.*, 2013).

#### Sample and Biochemical Tests

All isolated colonies were Gram stained and the following biochemical tests such as Coagulase, Catalase, oxidase, Indole, Citrate Utilization and Kligler's Iron Agar tests were conducted for their identification [6].

#### **Catalase Test**

Two (2) ml of hydrogen peroxide was measured into a sterile test tube, then a two colonies of the isolate was mixed in 3 ml of hydrogen peroxide using a sterile applicator stick, and was observed for effervescence or active bubbling [7].

#### Coagulase Test (Slide Method)

Normal saline (one drop) was carefully placed on both ends of a clean grease-free slide, followed by the addition of 2 colonies of the isolates using a sterile wire loop. The mixture was carefully mixed and a loopful of fresh human plasma was added to one end of the suspension, and and observed for clumping [7]. The slope and butt of the Kligler Iron Agar was inoculated with the isolates with the aid of a using a sterile straight wire loop and incubated at 35-37°C for 24hrs; The KIA was observed for lactose fermentation, hydrogen sulphide production and gas production [7]. Citrate utilization tests was carried out by aseptically inoculating the isolates using a sterile wire loop into the medium and incubated for 24 hours at 37°C. The medium was then observed for a blue colouration, indicating a positive reaction or no change in the colour of the medium indicating a negative reaction to citrate test [7]. Indole test was done by inoculating the isolates into tryptone broth and incubated at 37°C for 18 to 24 hours. Followed by addition of a few drops of Kovacs reagent and observing for red color ring within seconds an indication of a positive reaction [6]. Urease tests was done by inoculating the isolates into the urea agar and observing for orange to magnenta color as a positive reaction [7]. Oxidase test was carried out using commercial discs impregnated with the oxidase reagent. The isolates was smeared onto the disc with a sterile wooden stick. A deep purple color within 10 seconds indicated a positive reaction while the other colors or no change in color indicates a negative reaction [7].

## RESULTS

SAMPLE	COLONY COUNT	COLONY FORMING UNIT PER ML	LOG <sub>10</sub> (CFU/ML)
S1 NA	245	2.5x10 <sup>9</sup> CFU/ML	9.389166084
S1 NA(2)	130	1.3x10 <sup>9</sup> CFU/ML	9.113943352
S1 MAC	200	2.0x10 <sup>9</sup> CFU/ML	9.301029996
S1 MAC(2)	202	2.0x10 <sup>9</sup> CFU/ML	9.305351369
S1 SSA	237	2.4x10 <sup>9</sup> CFU/ML	9.374748346
S1 SSA(2)	241	2.4x10 <sup>9</sup> CFU/ML	9.382017043
S1 SAB	200	2.0x109CFU/ML	9.301029996
S1 SAB(2)	225	2.3x109CFU/ML	9.352182518
S2 NA	294	2.9x10 <sup>9</sup> CFU/ML	9.46834733
S2 NA(2)	290	2.9x10 <sup>9</sup>	9.462397998
S2 MAC	300	3.0x109CFU/ML	9.477121255
S2 MAC(2)	285	2.9x10 <sup>9</sup> CFU/ML	9.45484486
S2 SSA	300	3.0x109CFU/ML	9.477121255
S2 SSA(2)	250	2.5x10 <sup>9</sup> CFU/ML	9.397940087
S2 SAB	289	2.9x10 <sup>9</sup> CFU/ML	9.460897843
S2 SAB(2)	223	2.2x109CFU/ML	9.348304863
S3 NA	203	2.0x109CFU/ML	9.307496038
S3 NA(2)	275	2.8x10 <sup>9</sup>	9.439332694
S3 MAC	226	2.3x10 <sup>9</sup>	9.354108439
S3 MAC(2)	248	2.5x109CFU/ML	9.394451681
S3 SSA	225	2.3x109CFU/ML	9.352182518
S3 SSA(2)	328	3.3x10 <sup>9</sup> CFU/ML	9.515873844

 Table 1: Colony Forming Unit Per ML in Duplicate on Culture Media (DAY 1)

SAMPLE	COLONY COUNT	COLONY FORMING UNIT PER ML	LOG <sub>10</sub> (CFU/ML)
S3 SAB	155	1.6x10 <sup>9</sup> CFU/ML	9.11903317
S3 SAB(2)	178	1.8x10 <sup>9</sup> CFU/ML	9.250420002
S4 NA	217	2.2x10 <sup>9</sup>	9.336459734
S4 NA(2)	213	2.1x10 <sup>9</sup>	9.328379603
S4 MAC	123	1.2x109CFU/ML	9.089905111
S4 MAC(2)	259	2.6x109CFU/ML	9.413299764
S4 SSA	239	2.4x10°CFU/ML	9.378397901
S4 SSA(2)	294	2.9x10 <sup>9</sup> CFU/ML	9.46834733
S5 NA	233	2.3x10 <sup>9</sup>	9.367355921
S5 NA(2)	225	2.3x10 <sup>9</sup>	9.352182518
S5 MAC	228	2.3x10 <sup>9</sup> CFU/ML	9.357934847
S5 MAC(2)	218	2.2x10 <sup>9</sup> CFU/ML	9.338456494
S5 SSA	224	2.2x10 <sup>9</sup> CFU/ML	9.350248018
S5 SSA(2)	261	2.6x10°CFU/ML	9.416640507
S6 NA	200	2.0x10°CFU/ML	9.301029996
S6 NA(2)	289	2.9x10°CFU/ML	9.460897843
S6 MAC	123	1.2x10°CFU/ML	9.089905111
S6 MAC(2)	131	1.3x10°CFU/ML	9.117271296
S6 SSA	211	2.1x10°CFU/ML	9.324282455
S6 SSA(2)	206	2.1x10°CFU/ML	9.31386722
S7 NA	256	2.6x10°CFU/ML	9.408239965
S7 NA(2)		2.3x10°CFU/ML	
S7 MAC	227 156	1.6x10°CFU/ML	9.356025857 9.193124598
S7 MAC(2)	127	1.3x10 <sup>9</sup> CFU/ML	9.103803721
S7 SSA	226	2.3x10°CFU/ML	9.354108439
S7 SSA(2)	219	2.2x10 <sup>9</sup> CFU/ML	9.340444115
S7 SAB	118	1.2x10 <sup>9</sup> CFU/ML	9.071882007
S7 SAB(2)	105	1.1x10°CFU/ML	9.021189299
S8 NA	215	2.2x10 <sup>9</sup> CFU/ML	9.33243846
S8 NA(2)	218	2.2x10 <sup>9</sup> CFU/ML	9.338456494
S8 MAC	286	2.9x10 <sup>9</sup> CFU/ML	9.456366033
S8 MAC(2)	291	2.9x10 <sup>9</sup> CFU/ML	9.463892989
S8 SSA	234	2.3x10 <sup>9</sup> CFU/ML	9.369215857
S8 SSA(2)	229	2.3x10 <sup>9</sup> CFU/ML	9.359835482
S8 SAB	105	1.1x10 <sup>9</sup> CFU/ML	9.021189299
S8 SAB(2)	97	9.7x10 <sup>8</sup> CFU/ML	8.986771734
S9 NA	112	1.1x10 <sup>9</sup> CFU/ML	9.049218023
S9 NA(2)	109	1.1x10 <sup>9</sup> CFU/ML	9.037426498
S9 MAC	275	2.8x10 <sup>9</sup> CFU/ML	9.439332694
S9 MAC(2)	268	2.7x10°CFU/ML	9.428134794
S9 SSA	148	1.5x10°CFU/ML	9.170261715
S9 SSA(2)	146	1.5x10°CFU/ML	9.164352856
S9 SAB	194	1.9x10°CFU/ML	9.28780173
S9 SAB(2)	188	1.9x10 <sup>9</sup> CFU/ML	9.274157849
S10 NA	46	4.6x10 <sup>8</sup> CFU/ML	8.662757832
S10 NA(2)	58	5.8x10 <sup>8</sup> CFU/ML	8.763427994
S10 MAC	294	2.9x10 <sup>9</sup> CFU/ML	9.46834733
S10 MAC(2)	279	2.8x10 <sup>9</sup> CFU/ML	9.445604203
S10 SSA	203	2.0x10 <sup>9</sup> CFU/ML	9.307496038
S10 SSA(2)	187	1.9x10 <sup>9</sup> CFU/ML	9.271841607
S10 SAB	115	1.2x10 <sup>9</sup> CFU/ML	9.06069784

Keys: S- Sample; NA- Nutrient agar; MAC- MacConkey agar; SA- Salmonella Shigella agar; SAB- Sabouraud Dextrose agar

Table 1 depicts the total colony count of isolates in duplicates on various culture media, and the highest

colony count was  $3.0 \times 10^9$  CFU/ML on MacConkey and Salmonella Shigella Agar from Sample 2; while the

lowest was 4.6x10<sup>8</sup>CFU/ML on Nutrient Agar from Sample 10.

Table 2: Colony Forming Unit Per ML in Duplicate on Culture Media (DAY 3) SAMPLE **COLONY FORMING UNIT PER ML COLONY COUNT** LOG10 (CFU/ML) 2.9x109CFU/ML S1 NA 294 9.46834733 S1 NA(2) 213 2.1x109CFU/ML 9.328379603 S1 MAC 2.7x109CFU/ML 9.428134794 268 S1 MAC(2) 227 2.3x109CFU/ML 9.356025857 83 8.3x108CFU/ML 8.919078092 S1 SSA S1 SSA(2) 15 1.5x108CFU/ML 8.176091259 S1 SAB 86 8.6x108CFU/ML 8.934498451 92 S1 SAB(2) 9.2x108CFU/ML 8.963787827 292 S2 NA 2.9x109CFU/ML 9.465382851 S2 NA(2) 300 3.0x109CFU/ML 9.477121255 S2 MAC 185 1.9x109CFU/ML 9.267171728 9.338456494 2.2x109CFU/ML S2 MAC(2) 218 S2 SSA 236 2.4x109CFU/ML 9.372912003 S2 SSA(2) 185 1.9x109CFU/ML 9.267171728 S2 SAB 153 1.5x109CFU/ML 9.184691431 S2 SAB(2) 118 1.2x109CFU/ML 9.071882007 S3 NA 193 1.9x109CFU/ML 9.285557309 2.1x109CFU/ML S3 NA(2) 208 9.318063335 S3 MAC 286 2.9x109CFU/ML 9.456366033 2.2x109CFU/ML S3 MAC(2) 218 9.338456494 S3 SSA 171 1.7x109CFU/ML 9.23299611 S3 SSA(2) 112 1.1x109CFU/ML 9.049218023 222 S3 SAB 2.2x109CFU/ML 9.346352975 S3 SAB(2) 232 2.3x109CFU/ML 9.365487985 286 2.9x109CFU/ML 9.456366033 S4 NA S4 NA(2) 279 2.8x109CFU/ML 9.445604203 216 2.2x109CFU/ML 9.334453751 S4 MAC S4 MAC(2) 248 2.5x109CFU/ML 9.394451681 9.403120521 S4 SSA 253 2.5x109CFU/ML 9.428134794 S4 SSA(2) 268 2.7x109CFU/ML S4 SAB 222 2.2x109CFU/ML 9.346352975 2.2x109CFU/ML 9.340444115 S4 SAB(2) 219 2.8x109CFU/ML S5 NA 281 9.44870632 S5 NA(2) 278 2.8x109CFU/ML 9.444044796 S5 MAC 268 2.7x109CFU/ML 9.428134794 S5 MAC(2) 274 2.7x109CFU/ML 9.437750563 179 S5 SSA 1.8x109CFU/ML 9.252853031 S5 SSA(2) 174 1.7x10<sup>9</sup>CFU/ML 9.240549248 112 1.1x109CFU/ML 9.049218023 S5 SAB S5 SAB(2) 115 1.2x109CFU/ML 9.06069784 122 1.2x109CFU/ML 9.086359831 S6 NA S6 NA(2) 143 1.4x109CFU/ML 9.155336038 1.2x109CFU/ML S6 SSA 120 9.079181246 S6 SSA(2) 124 1.2x109CFU/ML 9.093421685 129 1.3x109CFU/ML 9.11058971 S7 NA S7 NA(2) 138 1.4x109CFU/ML 9.139879086 S7 MAC 145 1.5x109CFU/ML 9.161368003 153 1.5x109CFU/ML S7 MAC(2) 9.184691431 2.4x109CFU/ML S7 SSA 243 9.385606274 S7 SSA(2) 267 2.7x109CFU/ML 9.426511261 223 2.2x109CFU/ML 9.348304863 S7 SAB S7 SAB(2) 205 2.1x109CFU/ML 9.311753861 S8 NA 232 2.3x109CFU/ML 9.365487985 9.359835482 S8 NA(2) 229 2.3x109CFU/ML

SAMPLE	COLONY COUNT	COLONY FORMING UNIT PER ML	LOG10 (CFU/ML)
S8 MAC	215	2.2x10 <sup>9</sup> CFU/ML	9.33243846
S8 MAC(2)	244	2.4x10 <sup>9</sup> CFU/ML	9.387389826
S8 SSA	192	1.9x10 <sup>9</sup> CFU/ML	9.283301229
S8 SSA(2)	184	1.8x10 <sup>9</sup> CFU/ML	9.264817823
S8 SAB	215	2.2x10 <sup>9</sup> CFU/ML	9.33243846
S8 SAB(2)	129	1.3x10 <sup>9</sup> CFU/ML	9.11058971
S9 NA	193	1.9x10 <sup>9</sup> CFU/ML	9.285557309
S9 NA(2)	115	1.2x10 <sup>9</sup> CFU/ML	9.06069784
S9 MAC	194	1.9x10 <sup>9</sup> CFU/ML	9.28780173
S9 MAC(2)	207	2.1x10 <sup>9</sup> CFU/ML	9.315970346
S9 SSA	178	1.8x10 <sup>9</sup> CFU/ML	9.260071388
S9 SSA(2)	182	1.8x10 <sup>9</sup> CFU/ML	9.260071388
S10 NA	186	1.9x10 <sup>9</sup> CFU/ML	9.269512944
S10 NA(2)	184	1.8x10 <sup>9</sup> CFU/ML	9.264817823

Keys: S- Sample; NA- Nutrient agar; MAC- MacConkey agar; SA- Salmonella Shigella agar; SAB- Sabouraud Dextrose agar

Table 2 depicts the total colony count of isolates in duplicates on various culture media, and the highest colony count was 3.0x109CFU/ML on Nutrient Agar from Sample 2; while the lowest was 1.5x10<sup>8</sup>CFU/ML on Salmonella Shigella Agar from Sample 1.

Tab	Table 3: Colony Forming Unit Per ML in Duplicate on Culture Media (DAY 7)										
SAMPLE	COLONY COUNT	COLONY FORMING UNIT PER ML	LOG10 (CFU/ML)								
S1 NA	246	2.5x10 <sup>9</sup> CFU/ML	9.390935107								
S1 NA(2)	224	2.2x10 <sup>9</sup> CFU/ML	9.350248018								
S1 MAC	282	2.8x10 <sup>9</sup> CFU/ML	9.450249108								
S1 MAC(2)	287	2.9x10°CFU/ML	9.457881897								
S1 SSA	231	2.3x109CFU/ML	9.36361198								
S1 SSA(2)	236	2.4x109CFU/ML	9.372912003								
S1 SAB	122	1.2x109CFU/ML	9.086359831								
S1 SAB(2)	112	1.1x109CFU/ML	9.049218023								
S2 NA	111	1.1x109CFU/ML	9.045322979								
S2 NA(2)	163	1.6x10 <sup>9</sup> CFU/ML	9.212187604								
S2 MAC	128	1.3x109CFU/ML	9.10720997								
S2 MAC(2)	221	2.2x109CFU/ML	9.344392274								
S2 SSA	291	2.9x109CFU/ML	9.463892989								
S2 SSA(2)	284	2.8x109CFU/ML	9.45331834								
S2 SAB	218	2.2x109CFU/ML	9.338456494								
S2 SAB(2)	209	2.1x109CFU/ML	9.320146286								
S3 NA	148	1.5x10 <sup>9</sup> CFU/ML	9.170261715								
S3 NA(2)	139	1.4x10 <sup>9</sup> CFU/ML	9.1430148								
S3 MAC	264	2.6x10 <sup>9</sup> CFU/ML	9.421603927								
S3 MAC(2)	276	2.8x109CFU/ML	9.440909082								
S3 SSA	217	2.2x109CFU/ML	9.336459734								
S3 SSA(2)	204	2.0x10 <sup>9</sup> CFU/ML	9.31386722								
S3 SAB	166	1.7x10 <sup>9</sup> CFU/ML	9.220108088								
S3 SAB(2)	162	1.6x10 <sup>9</sup> CFU/ML	9.209515015								
S4 NA	215	2.2x109CFU/ML	9.33243846								
S4 NA(2)	227	2.3x109CFU/ML	9.356025857								
S4 MAC	119	1.2x109CFU/ML	9.075546961								
S4 MAC(2)	276	2.8x109CFU/ML	9.440909082								
S4 SSA	186	1.9x10 <sup>9</sup> CFU/ML	9.269512944								
S4 SSA(2)	174	1.7x10 <sup>9</sup> CFU/ML	9.240549248								
S4 SAB	192	1.9x10 <sup>9</sup> CFU/ML	9.283301229								
S4 SAB(2)	188	1.9x10 <sup>9</sup> CFU/ML	9.274157849								
S5 NA	222	2.2x10 <sup>9</sup> CFU/ML	9.346352975								
S5 NA(2)	124	1.2x10 <sup>9</sup> CFU/ML	9.093421685								

SAMPLE	COLONY COUNT	COLONY FORMING UNIT PER ML	LOG10 (CFU/ML)
S5 SSA	112	1.1x109CFU/ML	9.049218023
S5 SSA(2)	294	2.9x10 <sup>9</sup> CFU/ML	9.46834733
S6 NA	157	1.6x10 <sup>9</sup> CFU/ML	9.195899652
S6 NA(2)	174	1.7x10 <sup>9</sup> CFU/ML	9.240549248
S6 MAC	214	2.1x10 <sup>9</sup> CFU/ML	9.330413773
S6 MAC(2)	203	2.0x10 <sup>9</sup> CFU/ML	9.307496038
S6 SSA	200	2.0x10 <sup>9</sup> CFU/ML	9.301029996
S6 SSA(2)	192	1.9x10 <sup>9</sup> CFU/ML	9.283301229
S6 SAB	186	1.9x10 <sup>9</sup> CFU/ML	9.269512944
S6 SAB(2)	208	2.1x10 <sup>9</sup> CFU/ML	9.318063335
S7 NA	225	2.3x10 <sup>9</sup> CFU/ML	9.352182518
S7 NA(2)	212	2.1x10 <sup>9</sup> CFU/ML	9.326335861
S7 MAC	192	1.9x10 <sup>9</sup> CFU/ML	9.283301229
S7 MAC(2)	208	2.1x109CFU/ML	9.318063335
S7 SSA	168	1.7x10 <sup>9</sup> CFU/ML	9.225309282
S7 SSA(2)	179	1.8x109CFU/ML	9.252853031
S7 SAB	264	2.6x109CFU/ML	9.421603927
S7 SAB(2)	272	2.7x109CFU/ML	9.434568904
S8 NA	248	2.5x109CFU/ML	9.394451681
S8 NA(2)	237	2.4x109CFU/ML	9.374748346
S8 MAC	128	1.3x10 <sup>9</sup> CFU/ML	9.10720997
S8 MAC(2)	134	1.3x10 <sup>9</sup> CFU/ML	9.127104798
S8 SSA	182	1.8x109CFU/ML	9.260071388
S8 SSA(2)	179	1.8x109CFU/ML	9.252853031
S9 NA	116	1.2x109CFU/ML	9.064457989
S9 NA(2)	128	1.3x109CFU/ML	9.10720997
S9 MAC	211	2.1x109CFU/ML	9.324282455
S9 MAC(2)	176	1.8x109CFU/ML	9.245512668
S9 SSA	169	1.7x109CFU/ML	9.227886705
S9 SSA(2)	175	1.8x109CFU/ML	9.243038049
S9 SAB	207	2.1x109CFU/ML	9.315970346
S9 SAB(2)	229	2.3x109CFU/ML	9.359835482
S10 NA	101	1.0x10 <sup>9</sup> CFU/ML	9.004321374
S10 NA(2)	112	1.1x109CFU/ML	9.049218023
S10 MAC	176	1.8x10 <sup>9</sup> CFU/ML	9.245512668
S10 MAC(2)	174	1.7x109CFU/ML	9.240549248
S10 SSA	125	1.3x109CFU/ML	9.096910013
S10 SSA(2)	134	1.3x10°CFU/ML	9.127104798
S10 SAB	229	2.3x10 <sup>9</sup> CFU/ML	9.359835482
S10 SAB(2)	218	2.2x10 <sup>9</sup> CFU/ML	9.338456494

Keys: S- Sample; NA- Nutrient agar; MAC- MacConkey agar; SA- Salmonella Shigella agar; SAB- Sabouraud Dextrose agar

Table 3 depicts the total colony count of isolates in duplicates on various culture media, and the highest colony count was  $2.9 \times 10^9$  CFU/ML on Salmonella

Shigella Agar from Sample 5; while the lowest was  $1.0 \times 10^9$  CFU/ML on Nutrient Agar from Sample 10.

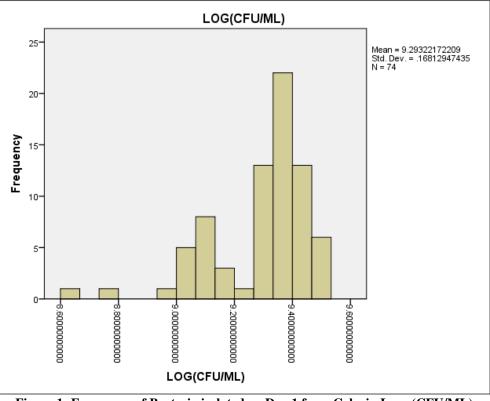


Figure 1: Frequency of Bacteria isolated on Day 1 from Cake in Log<sub>10</sub>(CFU/ML)

Figure 1 shows the graphical representation of data collected from the  $log_{10}(CFU/ML)$ . Where  $log_{10}(CFU/ML)$  values from 9.4 above shows the highest frequency, 8.6, 8.8, 9.0 and 9.2 were below 10 and 9.6

have no frequency at all. The mean value of  $log_{10}$ (CFU/ML) was 9.29322172209 or 1.96 x10<sup>9</sup>CFU/ML and standard deviation of 16812947435 in 74 isolates from 10 sampled cakes on day 1.

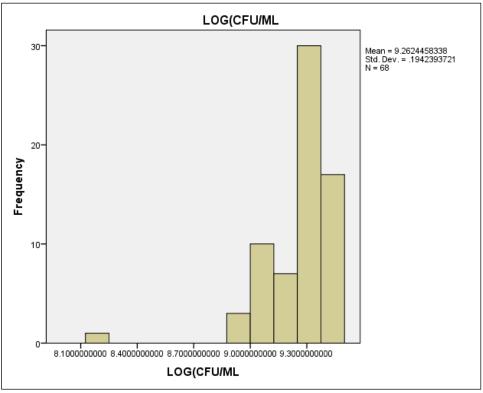


Figure 2: Frequency of Bacteria isolated on Day 3 from Cake in Log<sub>10</sub>(CFU/ML)

Figure 2 shows the graphical representation of data collected from the  $log_{10}(CFU/ML)$ . Where  $log_{10}(CFU/ML)$  values from 9.3 above shows the highest frequency, followed by 9.0. Ranges between 8.4 and 8.7 respectively have no frequency and 8.1 was below 10.

The mean value of  $log_{10}$ (CFU/ML) was 9.2624458338 or 1.83 x10<sup>9</sup>CFU/ML and standard deviation of 1942393721 in 68 isolates from 10 sampled cakes on day 3.

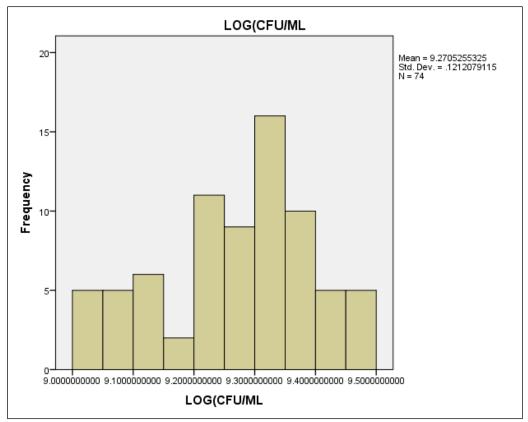


Figure 3: Frequency of Bacteria isolated on Day 7 from Cake in Log<sub>10</sub>(CFU/ML)

Figure 3 shows the graphical representation of data collected from the  $log_{10}(CFU/ML)$ . Where  $log_{10}(CFU/ML)$  values from 9.4 above shows the highest frequency, followed by ranges between 9.2 and 9.3 respectively. Ranges between 9.0, 9.1 and 9.5 have

frequency below 10. The mean value of  $log_{10}$ (CFU/ML) was 9.2705255325 or 1.86 x10<sup>9</sup>CFU/ML and standard deviation of 1212079115 in 74 isolates from 10 sampled cakes on day 7.

	Table 4. Oran reaction and Diochemical tests of Cake Isolates on Day 1										
Sample	Gram Reaction	Catalase	Coagulase	Citrate	Urease	Oxidase	Indole	Gas Pro.	H2S Pro.	Lactose Fer.	Suspected Organisms
S1	GP cocci	-	-	-	-	-	-	-	-	+	Streptococcus spp.
S1	GN Bacilli	+	-	+	-	+	_	-	I	-	Pseudomonas spp.
S1	GN Bacilli	+	-	I	-	-	+	+	I	+	Escherichia coli
S1	GP cocci	+									Candida albicans
S2	GP Bacilli	+	-	+	-	-	-	-	I	-	Bacillus spp.
S2	GN Bacilli	+	-	+	+	+	-	+	+	-	Proteus spp.
S2	GN Bacilli	+	-	-	-	-	+	+	1	+	Escherichia coli
S2	GP cocci	+									Candida albicans
S3	GN Bacilli	+	-	I	-	-	+	+	I	+	Escherichia coli
S3	GP cocci	+									Candida albicans
S4	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
S4	GN Bacilli	+	-	1	-	-	+	+		+	Escherichia coli
S5	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus

Table 4: Gram reaction and Biochemical tests of Cake Isolates on Day 1

Sample	Gram Reaction	Catalase	Coagulase	Citrate	Urease	Oxidase	Indole	Gas Pro.	H2S Pro.	Lactose Fer.	Suspected Organisms
S5	GN Bacilli	+	-	+	-	+	+	-	-	+	Aeromonas spp
S5	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S6	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S6	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
<b>S</b> 7	GP cocci	-	-	-	-	-	-	-	-	+	Streptococcus spp.
<b>S</b> 7	GN Bacilli	+	-	+	-	+	_	-	-	-	Pseudomonas spp.
S7	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S7	GP cocci	+									Candida albicans
<b>S</b> 8	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
<b>S</b> 8	GN Bacilli	+	-	+	+	+	-	+	+	-	Proteus spp.
<b>S</b> 8	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
<b>S</b> 8	GP cocci	+									Candida albicans
S9	GP Bacilli	+	-	+	-	-	-	-	-	-	Bacillus spp.
S9	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S9	GP cocci	+									Candida albicans
S10	GP cocci	+	-	-	-	-	-	-	-	+	Streptococcus spp.
S10	GN Bacilli	+	-	+	-	+	_	-	-	-	Pseudomonas spp.
S10	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S10	GP cocci	+									Candida albicans

S=Sample;GN=Gram Negative;GP=Gram Positive

Results from Table 4 shows Gram reaction and biochemical characterization of thirty two microorganisms isolated from ten (10) cake samples on DAY 1. The suspected microrganisms and number of cake samples they were isolated from are as follows: Aeromonas spp. [1] Bacillus spp. [2], Candida albicans [7], Escherichia coli(10), Proteus Spp. [2], Pseudomonas spp. [3], Staphylococcus aureus [3], Staphylococcus epidermidis [1] and Streptococcus spp. [3].

Sample	Gram Reaction	Catalase	Coagulase	Citrate	Urease	Oxidase	Indole	Gas Pro.	H2S Pro.	Lactose Fer.	Suspected Organisms
S1	GP Bacilli	+	-	+	-	-	-	-	-	-	Bacillus spp.
<b>S</b> 1	GN Bacilli	+	-	+	-	+	I	-	-	-	Pseudomonas spp.
<b>S</b> 1	GN Bacilli	+	-	-	-	-	+	+	-	+	Escherichia coli
S1	GP cocci	+									Candida albicans
S2	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
S2	GN Bacilli	+	-	+	+	+	1	+	+	1	Proteus Spp.
S2	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
S2	GP Yeast cells	+									Candida albicans
<b>S</b> 3	G P cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
<b>S</b> 3	GN Bacilli	+	-	-	-	-	+	+	-	+	Escherichia coli
<b>S</b> 3	GP Yeast cells	+									Candida albicans
S4	GN Bacilli	+	-	-	-	-	+	+	-	+	Escherichia coli
S4	GP Yeast cells	+									Candida albicans
S5	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S5	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S5	GP Yeast cells	+									Candida albicans
S6	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
S6	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
<b>S</b> 7	GP cocci	-	-	-	-	-	-	-	-	+	Streptococcus Spp.
<b>S</b> 7	GN Bacilli	+	-	+	-	+	_	-	-	-	Pseudomonas Spp.
S7	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.

 Table 5: Gram reaction and Biochemical tests of Cake Isolates on Day 3

Sample	Gram Reaction	Catalase	Coagulase	Citrate	Urease	Oxidase	Indole	Gas Pro.	H2S Pro.	Lactose Fer.	Suspected Organisms
S7	GP Yeast cells	+									Candida albicans
<b>S</b> 8	GN Bacilli	+	-	+	+	+	-	+	+	-	Proteus Spp.
<b>S</b> 8	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
<b>S</b> 8	GP Yeast cells	+									Candida albicans
<b>S</b> 9	GP cocci	-	-	-	-	-	-	-	-	+	Streptococcus Spp.
<b>S</b> 9	GN Bacilli	+	-	+	-	+	_	-	-	-	Klebsiella Spp.
<b>S</b> 9	GP Yeast cells	+									Candida albicans
S10	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus

S=Sample; GN=Gram Negative; GP=Gram Positive

Results from Table 5 shows Gram reaction and biochemical characterization of twenty nine microorganisms isolated from ten (10) cake samples on DAY 3. The suspected microrganisms and number of cake samples they were isolated from are as follows: Bacillus spp. [1], Candida albicans [8], Escherichia coli [5], Klebsiella Spp. [1], Proteus Spp. [2], Pseudomonas spp.(2), Salmonella Spp. [3], Staphylococcus aureus [3], Staphylococcus epidermidis [2], Streptococcus spp. [2].

Sample	Gram Reaction	Catalase	Coagulase	Citrate	Urease	Oxidase	Indole	Gas Pro.	H2S Pro.	Lactose Fer.	Suspected Organisms
<b>S</b> 1	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S1	GN Bacilli	+	-	-	-	-	+	+	_	+	Escherichia coli
S1	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.
S2	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
S2	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S2	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
S2	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.
<b>S</b> 3	GP diplococci	+	I	-	-	-	-	-			Micrococcus Spp.
<b>S</b> 3	GN Bacilli	+	I	+	-	I	+	I	+	I	Salmonella Spp.
<b>S</b> 3	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.
S4	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S4	GN Bacilli	+	-	-	-	-	+	+	I	+	Escherichia coli
S4	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.
S5	GP diplococci	+	I	+	-	+	+	-	-	+	Micrococcus Spp.
S5	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
S6	GP diplococci	+		+	-	+	+	-	-	+	Micrococcus Spp.
S6	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
S7	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.
S7	GN Bacilli	+	1	I	-	1	+	+	I	+	Escherichia coli
<b>S</b> 7	GP Yeast cells	+	I	I	-	I	I	I	I	I	Candida Spp.
S7	GN Bacilli	+	1	+	-	+	+	1	1	+	Aeromonas Spp
<b>S</b> 8	GN Bacilli	+	I	+	-	I	+	I	+	I	Salmonella Spp.
<b>S</b> 8	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
<b>S</b> 8	GN Bacilli	+	1	I	-	1	+	+	I	+	Escherichia coli
<b>S</b> 9	GP cocci	+	-	-	-	-	-	-	-	-	Candida Spp.
S10	GP cocci	+	+	-	-	-	-	-	-	-	Staphylococcus aureus
S10	GP cocci	+	-	-	-	-	-	+	-	+	Staphylococcus epidermidis
S10	GN Bacilli	+	-	+	-	-	+	-	+	-	Salmonella Spp.
S10	GP Yeast cells	+	-	-	-	-	-	-	-	-	Candida Spp.

Table 6: Gram reaction and Biochemical tests of Cake Isolates on Day 7

S=Sample; GN=Gram Negative; GP=Gram Positive

Results from Table 6 shows Gram reaction and biochemical characterization of twenty nine microorganisms isolated from ten (10) cake samples on DAY 7. The suspected microrganisms and number of cake samples they were isolated from are as follows: Aeromonas spp. [1], Candida albicans [8], Escherichia coli [4], Micrococcus (3) Salmonella Spp. [6], Staphylococcus aureus [2] and Staphylococcus epidermidis [5].

Bacteria	Source	Day 1- No of	Day 3- No of	Day 7- No of
		Isolates (%)	Isolates (%)	Isolates (%)
Bacillus spp	Cake	2(6.3)	1(3.5)	0(0)
Pseudomonas spp	Cake	3(9.4)	2(6.9)	0(0)
Escherichia coli	Cake	10(31.2)	5(17.2)	4(13.8)
Staphylococcus aureus	Cake	3(9.4)	3(10.3)	2(6.9)
Proteus spp	Cake	2(6.3)	2(6.9)	0(0)
Salmonella spp	Cake	0(0)	3(10.3)	6(20.7)
Candida albican	Cake	7(21.9)	8(27.6)	8(27.6)
Staphylococcus epidermidis	Cake	1(3.1)	2(6.9)	5(17.2)
Streptococcus spp	Cake	3(9.4)	2(6.9)	0(0)
Klebsiella spp	Cake	0(0)	1(3.5)	0(0)
Micrococcus spp	Cake	0(0)	0(0)	3(10.3)
Aeromonas spp	Cake	1(3.1)	0(0)	1(3.4)
Total		32(100)	29(100)	29(100)

### Table 7: Prevalance of Bacteria Isolated from Ten different Cakes

Table 7 shows the percentage and number of suspected bacteria identified from the cake samples collected from major bakers in Yenagoa metropolis. Out of thirty two microorganisms isolated organisms from the Day1 cake samples, the highest occurring microbes were *Escherichia coli* 10 (31.2%), followed by *Candida albicans* 7(21.9%), while *Staphylococcus epidermidis* and *Aeromonas spp* were the least in occurrence at 1(3.1%). On the Day 3, twenty nine microorganisms were isolated from same samples and *Candida albicanss* and *Escherichia coli* had the highest occurrence of 8(27.6%) and 5(17.2%) respectively; while *Bacillus Spp* and *Klebsiella spp* both had the lowest occurrence of 1(3.5%).

Twenty nine microorganisms were isolated on Day 7 and the highest occurring organism was *Candida spp* and *Salmonella spp* at 8(27.6%) and 6(20.7%) respectively; while *Aeromonas spp* and *Staphylococcus aureus* had the lowest occurrence of 1(3.4%) and 2(6.9%) respectively.

## DISCUSSION

This research recorded a highest colony count of  $3.0x10^9$ cfu/ml and lowest colony count of  $4.6x10^8$ cfu/ml from the cake samples in Day 1. Day 3 cake samples also recorded a highest colony count of  $3.0x10^9$ cfu/ml and a drop in the lowest colony count to  $1.5x10^8$ cfu/ml. However, the microbial contamination of the cakes reduced slightly by Day7 with highest and lowest total colony counts of  $2.9x10^9$ cfu/ml and  $1.0x10^9$ cfu/ml respectively. A similar study on Pukis (Indonesian) cake had highest total plate count of  $1.93x10^4$  Colonies per gram and lowest total plate count of  $1.19x10^4$  colonies/gram [8]. The storage containers, food handlers, and vending equipment may be the source of microbial contamination [9]; and the reduction in the total colony count on the cakes from Day 1 to Day 7 may be due to dessication. The percentage occurence of microorgamisms isolated from cakes on Day1, Day 3 and Day 7 were as follows: Aeromonas spp (3.1,0 and 3.4%); Bacillus spp (6.3,3.5 and 0%); Candida albicans (21.9,27.6 and 27.6%); Escherichia coli (31.2,17.2 and 13.8%); Klebsiella spp (0,3.5 and 0%); Micrococcus Spp (0,0 and 10.3%); Proteus spp (6.3,6.9 and 0%); Pseudomonas spp (9.4,6.9 and 0%); Salmonella spp (0, 10.3 and 20.7%); Staphylococcus aureus (9.4,10.3 and 6.9%); Staphylococcus epidermidis (3.1,6.9 and 17.2); Streptococcus spp (9.4,6.9 and 0%). The results of this study have some similar microbes as isolated by [10] from plain cakes from Eateries and Parties such as Bacillus laterosporus, Staphylococcus epiderdimis and Staphylococcus aureus, Aspergillus fumigatus, Rhizopus stolonifera, Penicillum chrysogenum, Aspergillus niger, Penicillum citrinum and Aspergillus flavus. Another study examined pathogenic bacteria in thirty cake samples and isolated Staphylococcus aureus in all cake samples except seven samples. Aeromonas specie was presented in twenty nine samples. Other isolates identified were Bacillus cereus, Bacillus sublilis, Pseudomonas spp., Echerichia spp., Salmonella spp., Shigella spp., Vibrio spp. and Micrococcus spp [11]. Another related study isolated *Enterobacter aerogenes*; Escherichia coli; Salmonella spp; Shigella spp; Staphylococcus aureus and Vibrio spp from Soy bean cake [12] A study in Tripoli to investigate the incidence of pathogenic bacteria in cake s and tarts revealed an incidence of S. aureus with 94.4 and 48.0 %, E. coli O157 with 14.7 and 4.0 % and Salmonella spp. with 5.9 and 8.0 % in cakes and tarts samples respectively [13].

From thirty two microorganisms isolated organisms from the Day1 cake samples, the highest occurring microbes were *Escherichia coli* 10 (31.2%), followed by *Candida albicans* 7(21.9%), while *Staphylococcus epidermidis* and *Aeromonas Spp* were the least in occurrence at 1(3.1%). On the Day 3, twenty nine microorganisms were isolated from same samples and *Candida albicans* and *Escherichia coli* had the highest occurrence of 8(27.6%) and 5(17.2%) respectively; while *Bacillus Spp* and *Klebsiella Spp* both had the lowest occurrence of 1(3.5%).

Twenty nine microorganisms were isolated on Day 7 and the highest occurring organism was *Candida spp* and *Salmonella spp* at 8(27.6%) and 6(20.7%) respectively; while *Aeromonas spp* and *Staphylococcus aureus* had the lowest occurrence of 1(3.4%) and 2(6.9%) respectively. *E.coli* was the highest occurring microbe from cake samples in Day 1 (31.2%), and second highest in Day 3 (17.2%); while *Candida albicans* was the second highest in Day 1 (21.9%), highest in Day 3 (27.6%) and highest in Day 7 (27.6%).

It is possible that most of the microbes were isolated from the cake samples from Day1 to Day 3 because they can survive at room temperature under a wide spectrum of environments; and due to their spore forming nature for others.

# CONCLUSION

The pathogenic microorganisms isolated in this were Escherichia coli, Candida albicans, study Salmonella Spp, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus Spp, Pseudomonas Spp, Proteus Spp, Bacillus Spp, Aeromonas Spp, and Micrococcus Spp; and this demonstrates the pending health hazards linked with these organisms via consuming contaminated cakes. This signifies that the cakes served in parties and major events are contaminated even before it reaches the consumer and that some of the organisms may be increasingly expressed as its days of storage increases. Therefore, there is an urgent need to educate bakers on the need to adopt standard baking methods and conditions to reduce and eradicate food poisoning and ill health by these microorganisms.

## RECOMMENDATION

Based on the above findings, the following is recommended:

- General and personal hygiene should be maintained by bakers.
- Bakers should pay more attention to their environment by fumigating regularly to avoid flies while they're working.
- Proper washing and cleaning of hands before and after baking and when necessary.
- Towels used during baking should be regularly and properly washed.

- Communication during baking should be avoided as much as possible.
- Health workers who are bakers should take their PPE and personal hygiene seriously to avoid contaminating the cakes with hospital acquired infections.
- Heath workers who are bakers should make sure they don't use the wears they have on in the hospital to the marketplace and their baking environment.

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