Bioscene

Bioscene
Volume- 21 Number- 02
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Bacteriological Assessment and Antibiogram of Persistent Bacterial Strains Found on Cutleries Used in Restaurants in Abakaliki Metropolis

Okonkwo Eucharia Chinyere[1], Onwa Ndubuisi Collins[2], Nwachi Anthonia Chinyere[1], Ogene Lilian Ngozi[1] & Nyah Oku-Abasi Etim[1]

[1]Department of Applied Microbiology, Faculty of Science, Ebonyi State University, P. M. B. 053 Abakaliki, Ebonyi State, Nigeria
[2]Department of Microbiology, Faculty of Biological Sciences, Alex Ekwueme Federal University, Ndufu Alike Ikwo, Ebonyi State, Nigeria

Corresponding Author: **Dr. Ugbo Emmanuel Nnabuike**

Abstract : Bacteria are ubiquitous in nature and their presence on cutleries used in restaurants cannot be underrated. The aim of this study was to assess the bacteriological and antibiogram of persistent bacterial strains found on cutleries used in restaurants in Abakaliki metropolis. A total of two hundred and eighty eight (288) swab samples were collected from cutleries used in different local and modern restaurants in Abakaliki. Bacteria identification was done using standard microbiological methods for isolation and characterization. Antibiogram was determined using the Kirby Bauer disk diffusion method. Multiple antibiotic resistance indices (MARI) were also determined. The results showed that four (4) bacteria genera were identified which includes Staphylococcus aureus 29 (20.1%), Escherichia coli 16 (11.1%), Salmonella species 15 (10.4%) and Klebsiella species 10 (6.9 %). Thus, Staphylococcus aureus and Klebsiella species showing the highest and lowest percentage frequency respectively in both modern and local restaurants visited. The isolates showed varying resistance to the tested antibiotics. These findings suggest eminent threat of food- borne diseases as well as other disease conditions. It also highlights the need for proper sterilization of cutleries, improved personal and environmental sanitary hygiene among restaurant owners and workers.

Key words: Bacteriological, antibiogram, persistent bacterial, cutleries

Introduction

Microbial attachment and biofilm formation to solid surface of cutleries provide some protection of the cells against physical removal of the cells by washing and cleaning of utensils (Orogu et al., 2017). Majority of these strain are associated with foodborne disease and can easily enter susceptible customer through use of contaminated spoon in the restaurants. Their presence can result in the recalcitrance and relapse of persistent bacterial infections, and it has been linked

to an increase in the risk of the emergence of antibiotic resistance during treatment (Fisher et al., 2017).

Risk of transmission is directly proportional to the duration of survival of the persistence bacteria strain on the colonized objects. The colonization and survival depends on geographical and environmental conditions like temperature, humidity, presence of organic matter, ability to form biofilms and the prevalent infection control practices (Bhatta et al., 2018). Persistent bacteria strain are a subpopulation of transiently antibiotic-tolerant bacterial cells that are often slow-growing or growth-arrested, and are able to resume growth after a lethal stress. The formation of persisted cells establishes phenotypic heterogeneity within a bacterial population and has been hypothesized to be important for increasing the chances of successfully adapting to environmental change (Fisher et al., 2017). The following are characteristic of persistent subpopulations: (i) cessation of cellular activity (dormancy), (ii) no growth or change in concentration in the presence of drug, (iii) no inherited persistence phenotype, and (iv) cells revert quickly to wild-type growth once the drug pressure is eliminated and nutrients are administered (Trastoy et al., 2018).

Bacteria utilize persistence and resistance to survive surfactant, disinfectant, antibiotic stress and environmental conditions (Jung et al., 2019; Trastoy et al., 2018). Also, evidence is accumulating that persisted cells can contribute to the emergence of antibiotic resistance. Persisted cells have been identified in every major pathogen, contributing to the antibiotic tolerance observed in biofilms, and are responsible for the recalcitrant nature of chronic bacterial infections (Defraine et al., 2018). A small fraction of transiently antibiotic-tolerant phenotypical variants, called persisted cells, is capable of surviving treatment with high doses of antibiotics. When antibiotic pressure drops, persisted cells that switch back to a normal phenotype can resume growth, ensuring survival of the bacterial population (Levin-Reisman et al., 2017; Trastoy et al., 2018; Defraine et al., 2018) in a susceptible host. However the survival of persistence bacteria strain on spoon is at least partly associated with the capacity of these microorganisms to detect and react to changes in environmental conditions.

These mechanisms enable an efficient, coordinated response to multiple stressors (Trastoy et al., 2018) and this feature may be acquired through exposure to environmental stress conditions (Trastoy et al., 2018) and applies only to bactericidal compounds. However, it has long been realized that tolerance and persistence, can also help bacteria to survive surfactant, disinfectant and antibiotic exposure (Levin-Reisman et al., 2017; Trastoy et al., 2018). Thus, little or no research has been done in the current area of study, therefore the need for the bacteriological assessment and antibiogram of persistent bacterial strains found on cutleries used in restaurants in Abakaliki metropolis in other to know their impact in public health.

Material and Methods

Collection of Specimens

A total of one hundred and forty four (144) samples each were both collected from different local and modern restaurants in Abakaliki amounting to a total of two hundred and eighty eight (288) samples. These items were sampled after the cleaning process was done on cutleries by the restaurant owner. For every restaurant, swab sticks were dipped inside a normal saline to swab the cutleries (eight cutleries from each of the restaurants were collected making a total of twenty four cutleries) in different restaurants and samples were properly labeled and transported within one hour of collection immediately to microbiology laboratory unit of Ebonyi State University.

Bacteriological Identification

The swab sticks containing the samples were suspended inside a sterilized nutrient broth and incubated (24 h) for 36 – 37°C to ascertain growth. After 24 h the medium showed a turbid growth and were immediately sub-cultured onto freshly prepared mannitol salt agar, MacConkey agar, and Salmonella/Shigella agar and incubated overnight (24 h) for 36 – 37°C. Then isolation was carried out for the test organism in which the isolates were subjected to different biochemical tests that are relevant in the identification of the bacteria.

Antibiotic Susceptibility Testing

In-vitro susceptibility testing was determined using the standard Kirby-Bauer disc diffusion technique. A loopful of each test bacterial isolates corresponding to 108 cells/ml was evenly streaked on Mueller-Hinton agar and the streaked plate was impregnated with different antibiotic discs manufactured by Oxoid Limited. The plates were all incubated at 37°C for 24h after which it indicated zones of inhibition and was carefully measured and interpreted as Resistant (R), and Sensitive (S) in conformity with the recommended standards established by the Clinical Laboratory Standards Institute (CLSI, 2017).

Determination of Multiple Antibiotic Resistance Index (MARI)

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate based on the interpreted results of the disk diffusion method analysis. The MAR index for a single isolate was calculated as the number of antibiotics to which an isolate is resistant (**a**) divided by the total number of antibiotics against which the isolate was tested (**b**) (Ugbo et al., 2023).

Results

Staphylococcus aureus was predominant with a frequency of 29 (20.1%), followed by Escherichia coli 16 (11.1%), Salmonella species 15 (10.4 %) and Klebsiella species showed the least distribution rate of 10 (6.9 %) (table 1). Staphylococcus aureus was also most frequency 26 (18.1 %) seconded by Salmonella species with

17 (11.8 %), Escherichia coli with occurrence rate of 12 (8.3 %) and Klebsiella species had the least distribution rate of 5 (3.5 %) (table 2). However, the overall frequency of bacteria showed high predominance of S. aureus 55 (38.1) over Salmonella species 32 (22.2), E. coli 28 (19.4 %), while the least predominant rate of 15 (10.4 %) were recorded against K.pneumoniae (table 3). Staphylococcus aureus showed 100% susceptibility to six different antibiotics such as gentimacin (100%), amikacin (100%), ciprofloxacin (100%), ceftriaxone (100%), minocycline (100%) and azithromycin (100%). E. coli was susceptible to four antibiotics which include gentamicin (100 %), ofloxacin (96.4 %), ciprofloxacin (92.9 %), minocycline (92.9 %) and resistant to amikacin (100 %), ceftazidime (100 %), azithromycin (100 %) and oxacillin (100%). Salmonella species showed 100 % susceptible to gentamicin, ofloxacin, ceftazidime, cefepime, ceftriaxone and minocycline and resistant to oxacillin (100 %), azithromycin (100 %) and ciprofloxacin (90.6 %). Klebsiella pneumoniae showed 100% susceptible gentamicin, amikacin, ofloxacin, ciprofloxacin, minocycline and 100 % resistant to ceftazidime, cefepime, ceftriaxone, azithromycin, and oxacillin (table 4). All the bacteria isolated strain demonstrated multidrug resistant with MARI value within the range of 0.3 - 0.6except Staphylococcus aureus as shown in Table 5.

Table 1: Distribution of Bacteria isolated from Cutleries used in local Restaurants

N=144				**Bacteria species**			
LOCATION 1	**AHIA OFURU MARKET**	**No of samples**	**E. coli (%)**	**S. aureus (%)**	**K. species(%)**	**Salmonella species (%)**	**Occurrence (%)**
	Knife	8	2(25)	2(25)	0(0.0)	1(12.5)	**5(62.5)**
	Spoon	8	1(12.5)	1(12.5)	1(12.5)	2(25)	**5(62.5)**
	Fork	8	0(0.0)	2(25)	1(12.5)	1(12.5)	**4(50)**
	Sub total	**24**	**3(12.5)**	**5(20.8)**	**2(8.3)**	**4(16.7)**	**14(58.3)**
LOCATION 2	**PRESCO**						
	Knife	8	1(12.5)	0(0.0)	0(0.0)	2(25)	**3(37.5)**
	Spoon	8	1(12.5)	2(25)	0(0.0)	1(12.5)	**4(50)**
	Fork	8	2(25)	1(12.5)	0(0.0)	0(0.0)	**3(37.5)**
	Sub total	**24**	**4(16.7)**	**3(12.5)**	**0(0.0)**	**3(12.5)**	**10(41.7)**
LOCATION 3	**KPIRIKPRI**						
	Knife	8	1(12.5)	2(25)	1(12.5)	0(0.0)	**4(50)**
	Spoon	8	0(0.0)	2(25)	1(12.5)	2(25)	**5(62.5)**
	Fork	8	1(12.5)	2(25)	2(25)	0(0.0)	**5(62.5)**
	Sub total	**24**	**2(8.3)**	**6(25)**	**4(16.7)**	**2(8.3)**	**14(58.3)**

Location 4	INT. MARKET	No of samples	E. coli (%)	S. aureus (%)	K. species (%)	almonella pecies (%)	Occurrence (%)
	Knife	8	0(0.0)	2(25)	0(0.0)	(25)	**4(50)**
	Spoon	8	1(12.5)	1(12.5)	0(0.0)	(12.5)	**3(37.5)**
	Fork	8	0(0.0)	2(25)	1(12.5)	(0.0)	**3(37.5)**
	Sub total	**24**	**1(4.1)**	**5(20.8)**	**1(4.1)**	**(12.5)**	**10(41.7)**
Location 5	**AZUGU**						
	Knife	8	2(25)	2(25)	0(0.0)	(12.5)	**4(50)**
	Spoon	8	0(0.0)	2(25)	2(25)	(0.0)	**4(50)**
	Fork	8	1(12.5)	2(25)	1(12.5)	(0.0)	**5(62.5)**
	Sub total	**24**	**3(12.5)**	**6(25)**	**3(12.5)**	**(4.1)**	**13(54.1)**
Location 6	**ISHIEKE**						
	Knife	8	2(25)	1(12.5)	0(0.0)	1(12.5)	**4(50)**
	Spoon	8	1(12.5)	1(12.5)	0(0.0)	1(12.5)	**3(37.5)**
	Fork	8	0(0.0)	2(25)	0(0.0)	(0.0)	**2(25)**
	Sub total	**24**	**3(12.5)**	**4(12.5)**	**0(0.0)**	**(8.3)**	**9(37.5)**
	Overall total	**144**	**16(11.1)**	**29(20.1)**	**10(6.9)**	**5(10.4)**	**70(48.6)**

Table 2: Distribution of Bacteria isolated from Cutleries use in Modern Restaurant

N=144				Bacteria species			
LOCATION	**A**	**No of samples**	**E. coli (%)**	**S. aureus (%)**	**K. pneumoniae (%)**	**Salmonella species (%)**	**Occurrence (%)**
	Knife	8	0(0.0)	2(25)	0(0.0)	0(0.0)	**2(25)**

	Spoon	8	2(25)	1(12.5)	0(0.0)	1(12.5)	**4(50)**
	Fork	8	0(0.0)	2(25)	0(0.0)	2(25)	**4(50)**
	Sub total	**24**	**2(8.3)**	**5(20.8)**	**0(0.0)**	**3(12.5)**	**10(41.7)**
LOCATION	**B**						
	Knife	8	1(12.5)	2(25)	1(12.5)	1(12.5)	**5(62.5)**
	Spoon	8	0(0.0)	2(25)	2(25)	0(0.0)	**4(50)**
	Fork	8	1(12.5)	2(25)	0(0.0)	2(25)	**5(62.5)**
	Sub total	**24**	**2(8.3)**	**6(25)**	**3(12.5)**	**3(12.5)**	**14(58.3)**
LOCATION	**C**						
	Knife	8	1(12.5)	1(12.5)	0(0.0)	2(25)	**4(50)**
	Spoon	8	2(25)	2(25)	0(0.0)	1(12.5)	**5(62.5)**
	Fork	8	1(12.5)	0(0.0)	1(12.5)	1(12.5)	**3(37.5)**
	Sub total	**24**	**4(16.7)**	**3(12.5)**	**1(4.1)**	**4(16.7)**	**12(50)**

LOCATION	**D**	**No of samples**	**E. coli (%)**	**S. aureus (%)**	**K. pneumoniae(%)**	**Salmonella species (%)**	**Occurrence (%)**
	Knife	8	0(0.0)	2(25)	0(0.0)	0(0.0)	**2(25)**
	Spoon	8	0(0.0)	2(25)	0(0.0)	0(0.0)	**2(25)**
	Fork	8	0(0.0)	1(12.5)	0(0.0)	2(25)	**3(37.5)**
	Sub total	**24**	**0(0.0)**	**5(20.8)**	**0(0.0)**	**2(8.3)**	**7(29.1)**
LOCATION	**E**						
	Knife	8	0(0.	1(12.5)	0(0.0)	0(0.0)	**1(12.5)**

			0)				
	Spoon	8	0(0.0)	2(25)	0(0.0)	0(0.0)	**2(25)**
	Fork	8	0(0.0)	0(0.0)	0(0.0)	1(12.5)	**1(12.5)**
	Sub total	**24**	**0(0.0)**	**3(12.5)**	**0(0.0)**	**1(4.1)**	**4(16.7)**
LOCATION	**F**						
	Knife	8	2(25)	1(12.5)	0(0.0)	0(0.0)	**3(37.5)**
	Spoon	8	2(25)	1(12.5)	1(12.5)	2(25)	**6(75)**
	Fork	8	0(0.0)	2(25)	0(0.0)	2(25)	**4(50)**
	Sub total	**24**	**4(16.7)**	**4(16.7)**	**1(4.1)**	**4(16.7)**	**13(54.1)**
	Overall total	**144**	**12(8.3)**	**26(18.1)**	**5(3.7)**	**17(11.8)**	**59(40.9)**

Table 3: Overall Frequency of Bacteria Isolates from Local Restaurant and Modern Restaurant

Organisms	**Local Restaurant (%)**	**Modern Restaurant (%)**
S. aureus	29 (41.4)	26 (43.3)
Salmonella spp	15 (21.4)	17 (28.3)
Escherichia coli	16 (22.9)	12 (20)
Klebsiella pneumoniae	10 (14.2)	5 (8.3)
Total	**70**	**60**

Table 4: Antibiotic susceptibility profile of bacteria isolates from local restaurant and modern restaurant

Antibiotics	**S. aureus**		**E. coli**		**Salmonella spp**		**K. pneumoniae**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Gentamicin	0 (0.0)	55(100)	0 (0.0)	28 (100)	0 (0.0)	32(100)	0 (0.0)	15(100)
Amikacin	0 (0.0)	55(100)	28(100)	0 (0.0)	2 (6.3)	30(93.8)	0 (0.0)	15(100)

Ofloxacin	4 (7.3)	51(92.7)	1 (3.7)	27(96.4)	0 (0.0)	32(100)	0 (0.0)	15(100)
Ciprofloxacin	0 (0.0)	55(100)	2 (7.1)	26(92.9)	29(90.6)	3 (9.4)	0 (0.0)	15(100)
Ceftazidime	3 (5.5)	52(94.5)	28 (100)	0 (0.0)	0 (0.0)	32(100)	15(100)	0 (0.0)
Cefepime	2 (3.6)	53(96.4)	27(96.4)	1 (3.7)	0 (0.0)	32(100)	15(100)	0 (0.0)
Ceftriaxone	0 (0.0)	55(100)	24(85.7)	4 (14.3)	0 (0.0)	32(100)	15(100)	0 (0.0)
Azithromycin	0 (0.0)	55(100)	28 (100)	0 (0.0)	32(100)	0 (0.0)	15(100)	0 (0.0)
Oxacillin	3 (5.5)	52(94.5)	28 (100)	0 (0.0)	32(100)	0 (0.0)	15(100)	0 (0.0)
Minocycline	0(0.0)	55(100)	2 (7.1)	26(92.9)	0 (0.0)	32(100)	0 (0.0)	15(100)

Key: R-Resistant; S-Susceptible

Table 5: Multiple antibiotic resistance index (MARI) of the isolated resistant bacteria from local and modern restaurants in abakaliki metropolis

Isolates	**(MARI)**
Klebsiella pneumoniae	0.5
Staphylococcus aureus	-
Escherichia coli	0.6
Salmonellaspecies	0.3
MEAN	**0.5**

Discussion

As noted in this study, four (4) genera of bacteria namely; Staphylococcus aureus, E. coli, Klebsiella pneumonia and Salmonella species were identified. These bacteria are of medical importance associated with different pathologic and syndrome of diseases. Their presence in both local and modern restaurant cutleries reiterate with other studies that reported their presence on cutleries and other kitchen items (Orogu et al., 2017; Afunwa et al., 2018). Frequent report of these bacteria on kitchen utensil may also reveal the persistent nature of these bacteria as most strain is able to produce biofilm on abiotic surfaces.

Local restaurants had higher proportion of bacteria, 70(48.6 %) over modern restaurants, 60 (41.7 %). The high predominance of bacteria in local restaurant could be accrued to poor personal hygiene practices amongst some food vendors which further accelerate the rate of contamination. Most of the local restaurants operate a "pay as you eat system" thus, exchange of money between the "buyers" and the "sellers" with unprotected hands and minimal sanitary

precautions presents as predisposing factors (Anyanwu et al., 2016; Afunwa et al., 2018). Additionally, most of the local restaurants were found in open areas where human activities are eminent, with increase airborne contamination due to dust and aerosols. On the centrally, low bacterial densities observed in modern restaurants could stem from the fact that cutleries were kept in closed baskets or trays that are not openly prone to contamination with bacteria in the open air. Besides, these modern restaurants operate in enclosed room with notable hygiene and orderliness.

Also, most local restaurant vendors handle vegetables and other food items with bare hands; which may encourage the transfer of pathogens in items especially with those uncooked foods like vegetable salad (Anyanwu et al 2016). Sponge and towel provide an ideal environment for bacteria to grow. Notably, wet towels can harbor potentially harmful organisms and become breeding grounds for bacteria (WHO, 2002).The use of towels in kitchen can cause the spread of bacteria to hands, equipment, cookery, and cutlery (WHO, 2002; Orogu et al., 2017).

The noted low isolation rate of bacteria in modern restaurants may also result from the use of dish washer as they aid in effective cleaning and drying of plate, especially on a hot wash cycle where temperatures can reach 75 °C (167 °F) (Suzy, 2015) which may denature most bacteria DNA.The undoubtedly presence of these bacteria may be typically transmitted by way of vectors, in most instance insects (cockroaches, ants, rat's mice). These vectors serve as the reservoir host which harbors various organisms that inflict illnesses. Studies conducted in Texas and Nigeria indicated that cockroaches were an important vector in pathogen spread due to their unsanitary lifestyle (Pechal et al., 2007 Akinjogunla et al., 2012).

In both local and modern restaurant S. aureus was the most predominant bacteria recording 41.4 % and 43.3 % respectively. S. aureus found in these items may be through handling since the bacteria is a normal flora of human skin. The presence of this organism in cutleries is of public health importance's because it usually responsible for staphylococcal food poisoning, endocarditis, sinusitis, skin infections such abscesses (Orogu et al., 2017)

Salmonella species was the second most predominant bacteria 21.4 %. Ingestion of cross-contamination of food containing these organisms poses health hazard to the consumers and may lead to deleterious effects (Ugbo et al., 2023). The occurrence of the aforementioned organisms may depends on the magnitude and range of the human activities/animal sources that release pathogens to the environment through animal waste use as plant manure as well as the level of treatment given to the water. The microbes may break through inadequate water treatment process. The overall presence of Escherichia coli is 28 (19.4 %). The organism is the most pathogenic organism found in the urinary tract of humans and is one of the major organisms that are implicated in wound infection, meningitis and bacteremia in neonates (Enitan et al., 2020). Klebsiella

pneumoniae accounted for 15 (10.4 %) of isolates and is the least isolated bacteria observed in this study. Klebsiella pneumoniae found in the samples is widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water. It has become a very important cause of nosocomial infection. It causes pneumonia, urinary infection, other pyogenic infections, septicemia, meningitis and rarely diarrhoea. K. pneumoniae has been implicated in oral infection because of its ability to degrade proteinaceous substances in the mouth resulting in bad breath (Iroha et al., 2022; Meinen et al., 2021; Enitan et al., 2020). Some strains of K. pneumoniae have been shown to produce an enterotoxin. In this study, the antibiotic susceptibility partern of the most recurrent organism, Staphylococcus aureus, agrees with the findings of Azim et al. (2011). Both studies reported high susceptibility of S. aureus to gentamicin (100%) and ciprofloxacin (100% and 80%) respectively. Also various degree of resistance to aforementioned antibiotics were reported (Farzana et al., 2011)

Conclusion

This study reports the presence of Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Salmonella species, E. coli, Klebsiella pnuemoniae) on cutleries. Local restaurant had high proportion of bacteria 70 (48.6 %) over modern restaurant 60 (41.7 %). Crowding, wetness of work surfaces and improper cleaning of kitchen equipment such as chopping boards, plates, knives, pots, bowls, mixers, refrigerators and other complex appliances like food processors, blenders and eggbeaters serve as good reservoir for these bacteria. Dish towels, hand towels, scrubbing sponges, garbage bins, sink drains and P-trap (the J-shaped pipe under the sink) are also not left out. The P-trap retains water over time and possibly seeps back up through the sink which may also enhance the spread of pathogens. Since these bacteria are major etiology of disease in human and also demonstrate MDR phenotype, judicious use of minocycline, ofloxacin and gentamicin for case management is important.

Competing Interests

The authors have not declared any conflict of interests.

References

1. Afunwa, R. A., Igwe, G. O., Afunwa, E. C., Ezebialu, C.U., Unachukwu, M. N and Okoli, C. E (2018). Bacteriological Examination of Utensils and Hands of Food Vendors in aUniversity Cafeteria in Enugu, Nigeria. Journal of Biology and Life Science, 10:1-4.
2. Anyanwu, N.C.J., John, W.C. and Idoko, M. O. (2016) Bacteriological Examination of the Cafeteria Equipments in Karu L.G.A Cafeterias, Nasarawa State, Nigeria. Journalof Applied Life Science International, 5(2),1

3. Akinjogunla, O. J., Odeyemi, A and Udoinyang, E (2012). Cockroaches (Periplaneta americana and Blattella germanica): reservoirs of multi drug resistant (MDR) bacteria in Uyo, Akwa Ibom state, Science Journal of Biological Science, 1:19–30.
4. Bhatta, D. B., Hamal, D., Shrestha, R., Subramanya, S. H., Baral, N., Singh, R. K., Nayak, N and Gokhale, S (2018). Bacterial Contamination of Frequently Touched Objects in a Tertiary Care Hospital of Pokhara, Nepal: How Safe are Our Hands? Antimicrobial Resistance and Infection Control, **7**:97-101.
5. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing M100S, 26th Edition. 2017.
6. Defraine, V., Fauvart, M and Michiels, J (2018). Fighting Bacterial Persistence: Current and Emerging Anti-persister Strategies and Therapeutics. Drug Resistance Updates, 38:12-26.
7. Enitan, S. S., Oluremi, A. S., Ochei, J. O., Akele, R. Y., Usiobeigbe, S. O., Emmanuel, I., Enitan, C. B. and Tajudeen, R. O (2020). Assessment of Oral Bacterial Profile and Antibiogram of Patients Attending Dental Clinic of a Private Tertiary Hospital in Ogun State, Nigeria. Saudi Journal of Oral and Dental Reserach, **5**(1): 11-23.
8. Farzana, K., Akram, M. R. and Mahmood, S (2011). Prevalence and antibiotics susceptibility patterns of some bacterial isolates from a street vended fruit product, African Journal of Microbiology Research, 5(11):1277-1284.
9. Fisher, R. A., Gollan, B. and Helaine, S. (2017). Persistent Bacterial Infections and Persister Cells.Nature Reviews Microbiology, 12:23-4
10. Iroha, I. R., Mohammed, I. D., Moses, I. B., Ngwu, N. J., Uzoeto, H. O., Oladimeji, A. S., Ikemesit, U. P., Onuora, L. A., Ewa, N. J and Edemekong, C.I (2022). Molecular Characterization of Enterobacteriacea Isolated from Gingivitis and Periodontitis Patients and the Antimicrobial Activity of Mouth Wash Agents. Scientific African, 15:1-106.
11. Jung, S., Ryu, C. and Kim, J. (2019). Bacterial Persistence: Fundamentals and Clinical Importance. Review Journal of Microbiology, 57(10):829-835.
12. Levin-Reisman, I., Ronin, I., Gefen, O., Braniss, I., Shoresh, N and Balaban, N. Q (2017). Antibiotic Tolerance Facilitates the Evolution of Resistance. Science, 355:826–830.
13. Lynn, M. and Nandita, D. (2010). Bacterial Contamination of Crockery and Cutlery within the Kiosks' Restaurants of the Federal University of Technology, Yola. African Journal of Microbiology Research, 4 (3):147-153.
14. Meinen, A., Reuss, A., Willrich, N., Feig, M., Noll, I., Eckmanns, T., Al-Nawas, B and Markwart, R (2021). Antimicrobial Resistance and the Spectrum of Pathogens in Dental and Oral-MaxillofacialInfections in Hospitals and Dental Practices in Germany. Frontier in Microbiology, 12:676-108
15. Neopane, P., Nepal, H. P., Shrestha, R., Uehara, O. and Abiko, Y. (2018). In vitro Biofilm Formation by Staphylococcus aureus Isolated from Wounds of

Hospital-admitted Patients and their Association with Antimicrobial Resistance. Journal of General Internal Medicine, 11:25–32.

16. Pechal, J. L., Austin, J., Gold, R and Tomberlin, J. K (2007) Epidemiology and Spatial Relationship of Bacteria Associated with Periplaneta Americana (Blattodea: Blattidae) in Central Texas, Journal of Agricultural and Urban Entomology, 24(4): 205-21
17. Polyzou, A., Slavakis, A and Pournaras, S (2001). Predominance of a MRSA Clone Susceptible to Erythromycin and Several other Non-Beta Lactam Antibiotics in a Greek Hospital. Journal of Antimicrobial Agents and Chemotherapy, 34(48): 231-23
18. Orogu, J. O., Ehiwario, N. J. and Adebisi, O. O. (2017). Microbiological assessment of cutleries. Moj Bioequiv. And Bioavailab. 3(6):159-162
19. Suzy, S (2015). "You Should Pretty Much NEVER Wash Your Dishes By Hand. Here's Why". Huffington Post. Retrieved 2017-12-3
20. Trastoy, R., Manso, T., Fernández-García, L., Blasco, L., Ambroa, A., Pérez del Molino, M. L., Bou, G., García-Contreras, R., Wood, T. K and Tomás, M (2018). Mechanisms of Bacterial Tolerance and Persistence in the Gastrointestinal and Respiratory Environments. Clinical Microbiology Review,31:23-18.
21. Ugbo, E. N., Jacob, J. I., Effendi, M. H., Witaningrum, A. M., Agumah, B. N., Ugbo, A. I. and Moses, B. I. (2023). Poultry slaughterhouse wastewater as reservoirs for spreading extended-spectrum beta-lactamase-producing Escherichia coli in Abakaliki, Nigeria. Biodiversitas, 24(9): 4960-4966.
22. Ugbo, E. N., Effendi, M. H., Witaningrum, A. M., Tyasningsih, W., Agumah, B. N., Ugbo, A. I., Nnabugwu, C. C. and Okata-Nwali, D. O. (2023). Antimicrobial resistance pattern of Salmonella spp. isolated from poultry farms in Abakaliki, Nigeria. Biodiversitas, 24(9): 5207-5214.
23. WHO. Bulletin of the World health Organization. Food Safety matters, Switzerland: Geneva; 2002.