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Bacteriological Analysis and Plasmid Profiles of Surfaces of Some Hospital Kitchen Equipment in Benin City, Nigeria

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ABSTRACT: The surfaces of hospital kitchen equipment could be a major source of transmission of resistant pathogens to patients. Hence, the objective of this paper was to assess the bacteriological and plasmid profiles of surfaces of tables, sinks, chopping-boards, gas cookers and freezer handles hospital kitchen equipment in Benin City, Nigeria using appropriate standard microbiological techniques. Bacterial plasmids were isolated and separated using the agarose gel electrophoresis. Plasmid curing was performed using acridine orange. The antimicrobial sensitivity pattern showed that the bacterial isolates exhibited varying degree of resistance to the antibiotics. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were highly resistant to the antibiotics, having multiple antibiotic resistances (MAR) index of 0.6. *Micrococcus* spp. and *Klebsiella pneumoniae* were the least resistant to the antibiotics. Plasmid analysis revealed the presence of single and multiple-banded plasmids with sizes ranging from 100-1000bp. After curing, all the isolates (except *S. aureus*, *E. coli* and *P. aeruginosa*) were sensitive to all the antibiotics, indicating a significant reduction in antibiotic resistance after curing. The results revealed that plasmids played a significant role in conferring resistance on the isolates. Regular cleaning and disinfection should be strictly observed in hospital kitchens to prevent outbreaks and spread of resistant pathogens in hospitals.

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The hospital environment is a significant reservoir of pathogens for transmission to patients in many ways including hospital kitchen and equipment surfaces. Hospital kitchen equipment host relatively diverse microbial communities, mainly dominated by bacteria (Ghita *et al*., 2020). Bacteria readily colonize kitchen equipment and surfaces, and the exchange of microbes between humans and the kitchen environment can impact human health. It has also been reported that poor handling of hospital kitchen equipment and maintenance of hospital kitchen equipment have played significant role in the spread of microorganisms in hospital kitchen environments (Burge *et al*., 2000). Microorganisms have been reported to contaminate hospital kitchen tools. This has led to the proliferation of microorganisms and result to infectious diseases. Outbreaks of infections associated with contact surfaces and inanimate objects are caused by items that should be sterile but have become contaminated with pathogenic microorganisms. The surfaces near colonized patients or those touched by food handlers can become contaminated by antibiotic resistant bacteria like *Escherichia coli*, *Staphylococcus aureus* and

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Pseudomonas aeruginosa (Ghita *et al*., 2020). It has been reported that disinfection techniques are sometimes incapable of eradicating fomite reservoirs of nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA). This is a major public health concern because antibiotic resistant bacteria are increasingly responsible for higher morbidity and mortality rates from nosocomial infections (Worku *et al*., 2018).

The use of antimicrobial agents in clinical settings to treat infectious diseases as well as their use in agriculture is of great concern to public health, as this can lead to the development and evolution of antibiotic resistant bacteria. This occurs as a result of the selective pressure that antibiotics place on bacteria, resulting in the proliferation and dissemination of resistant bacteria. Resistance genes are transferred from bacteria to bacteria via plasmids and other mobile genetic elements, thereby resulting in the spread of multidrug resistant bacteria (Kunhiraman *et al*., 2023). Therefore, the objective of this paper was to assess the bacteriological and plasmid profiles of surfaces of tables, sinks, chopping-boards, gas cookers and freezer handles hospital kitchen equipment in Benin City, Nigeria.

MATERIALS AND METHODS

Sample Collection: The samples used for this study was collection from the kitchen of three major hospitals in Benin City, Edo State, Nigeria. Sterile swab sticks moistened in 2ml sterile saline solution were used for sample collection. Multiple samples were collected aseptically from tables, sinks, gas cookers, chopping boards and freezer handle surfaces. They were immediately taken to the laboratory for microbiological analysis.

Culture and Identification of Isolates: The media used in this study were nutrient agar (Oxoid) for purification and storage in slants, MacConkey agar (Oxoid) for isolation of Gram-negative bacteria, eosin-methylene blue (EMB) agar for selective isolation of *Escherichia coli*, blood agar and mannitol salt agar (MSA) for isolation of *Staphylococcus aureus*. The media were prepared according to the manufacturer's prescription and poured onto sterile petri plates. The media were sterilized by autoclaving at 121^{o} C for 15 mins at 15 Psi and allowed to cool at about 45^{o} C before been poured onto sterile petri plates.

The samples were plated onto the prepared culture plates and incubated at 37^{o} C for 24 h. After incubation, colonies having different morphology, shape and size were sub-cultured until pure cultures were obtained. Each colony was gram stained, and identification was done by morphological and biochemical methods (Cheesbrough, 2006).

Antibiotics Susceptibility Testing: Antibiotics susceptibility testing was carried out using the disc diffusion (Kirby-Bauer) technique, as recommended by standard guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015). The media used were Mueller Hinton broth and Mueller Hinton agar (Lab M, Lancashire, UK). Five (5) colonies of the test bacteria from culture medium were inoculated into Mueller Hinton broth and incubated at 37^{o} C for 24 h. Using sterile syringe and needle, 0.5ml of the culture was transferred onto the surfaces of Mueller Hinton agar and spread evenly by gently rotating the plates. Using sterile forceps, the antibiotic discs were placed appropriately and evenly on the inoculated plates. The plates were incubated at 37°C for 24 h.

The following commercially available antibiotic discs (Mast Diagnostics, Merseyside, United Kingdom) were used for susceptibility testing: cotrimoxazole (SXT) 30µg, penicillin (PN) 30µg, amoxicillin (AM) 30µg, chloranphenicol (CH) 30µg, gentamicin (CN) 10µg, ciprofloxacin (CPX), pefloxacin (PEF) 30µg, augmentin (AU) 30µg, tarivid (OFX), streptomycin (S) 30µg and sparfloxacin (SP) 10µg. The diameters of the zones of inhibition (in millimetres) were measured using a meter rule, and it was interpreted as sensitive, intermediate and resistant using the CLSI standard (CLSI, 2015).

The multiple antibiotic resistance (MAR) index was calculated as the ratio between the number of antibiotics that an isolate is resistant to and the total number of antibiotics the organism is exposed to. A MAR index greater than 0.2 indicated high risk source of contamination and its of public health significance (Krumperman, 1983).

Plasmid Profile of the Bacterial Isolates: Bacteria that showed resistance were subjected to plasmid profiling. A colony of test organisms, cultured in fresh agar plate was picked with the aid of a sterile wire loop and inoculated into sterile test tubes containing 8 ml of fresh nutrient broth and then inoculated at 37 ^{o}C for 72 h. The antibiotic resistance plasmid in the cultures were isolated using the alkaline lyse protocol (Birnboim and Doly, 1979).

Agarose gel (0.8%) was prepared by weighing out 0.8 g of agarose and dissolving in 100 ml of 1x TBE (Tris-Boric Acid-EDTA buffer) with the aid of heat. A standard DNA ladder was also loaded in a hole between the sample plasmid preparations. The tray with the gel was buffered with TBA buffer and the

plasmid preparation from the different bacterial isolates, added to the different holes was set up to migrate towards the positive charged electrode. The gel was allowed to run for 3 h at 63 V. The gel containing the separated plasmid was removed and visualized under UV light with the aid of UV goggle. A picture of the DNA was taken showing size and mobility on the agarose gel. The size (mm) was determined relative to the standard DNA ladder loaded in between the sample plasmid preparation (Birnboim and Doly, 1979).

Plasmid Curing: Curing of plasmids was performed by the method of Zaman *et al*. (2010), using three different concentrations acridine orange (50 µg/ml, 75 µg/ml, 100 µg/ml). Amoxicillin resistance was used as the selectable marker. An overnight culture of each test organism in Luria Broth (LB) containing amoxicillin was diluted to 10^4 cells/ml using freshly prepared sterile LB by serial dilution technique. After 24 h incubation at 37°C, plates were observed for the cured cells. The cured plasmid cells were detected comparing the development of bacterial colonies on antibiotic containing plate with that of the normal (without antibiotic) plate. The samples that showed colonies on normal LB agar but failed to grow on LB agar supplemented with amoxicillin were the possible cured isolates.

Post-Curing Susceptibility Testing: The standardized inocula of the bacteria were swabbed on the Mueller Hinton agar plates. Then sterile forceps was used to impregnate the antibiotics disc on the plates. The plated were incubated at 37°C for 24 h and the diameters of the zones of inhibition were taken in millilitres (mm) (CLSI, 2015).

Statistical Analysis of Data: The data obtained from this research were analyzed using statistical package for social scientist (version 21), and Microsoft excel (version 2019). Values were expressed in tables and as mean at 0.05 significance levels (Ogbeibu, 2015).

RESULTS AND DISCUSSION

Prevalence of Bacterial Isolates: The results of this study showed the presence of Gram-positive bacteria: *Bacillus cereus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus* spp., and Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and Klebsiella *pneumoniae* on the kitchen equipment surfaces (Table 1). *Staphylococcus aureus* (28.6%) and *Escherichia coli* (16.0%) had the highest occurrence. The least occurrence was observed in *Enterobacter* spp. (2.5%).

Table 1: Percentage Occurrence of the Bacterial Isolates

Bacterial Isolates	Occurrence	Percentage (%)
Gram-Positive Bacteria		
B. cereus	17	14.3
S. aureus	34	28.6
S. epidermidis	16	13.4
Micrococcus spp.	7	5.9
Gram-Negative Bacteria		
E. coli	19	16.0
P. aeruginosa	12	10.1
Enterobacter spp.	3	2.5
K. pneumoniae	11	9.2
Total	**119**	**100**

This results obtained in this study revealed that the surfaces of the hospital equipment were contaminated with the organisms. This study correlates with the works of Anthony and Ogedegbe (2018) that isolated *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae and Bacillus cereus* from hospital environmental surfaces in Akure, Nigeria. Also, Shayeghi *et al*. (2020) isolated eight bacterial strains: *Staphylococcus aureus*, *S. epidermidis*, *E. coli*, *Shigella* spp., *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* and *Enterococcus* spp., from kitchen instruments in Tehran (Iran). They concluded that bacteria could colonise surfaces of washed and uncleaned kitchen utensils which may have a high substantial role in transferring such microorganisms to other surfaces and furthermore cause food poisoning and food-borne diseases.

Antibiotic Susceptibility Testing: Table 2 shows antibiotic susceptibility tests of the isolates. All the Gram-positive bacteria were sensitive to erythromycin. Of the Gram-positive bacteria, *Staphylococcus aureus* was most resistant, it was resistant to 6 out of the 10 antibiotics tested and had MAR index of 0.6. *Micrococcus* spp. was the least resistant with MAR index of 0.3. All the Gram-negative bacteria were resistant perfloxacin, amoxicillin and augmentin, but were sensitive to cotrimoxazole, ofloxacin and chloramphenicol. *Escherichia coli* and *Pseudomonas aeruginosa* were the most resistant with MAR index of 0.6. *Klebsiella pneumoniae* was the least resistant with MAR index of 0.4. From the results of the antibiotic susceptibility tests, most of the antibiotics were not effective in the treatment of the bacterial isolates. This result is in concordance with the studies of Aminu *et al*. (2014) who reported that all the isolates (including *S. aureus* and *S. epidermidis*) from fomites in a teaching hospital in Northern Nigeria were sensitive to erythromycin. The resistance of *S. aureus* to gentamycin, ampiclox, zinnacef and cotrimoxazole is also in agreement with the report by Wolde *et al*. (2015). Nguyen *et al*., 2024 reported that *S. aureus* isolated from *S. aureus*

infections from 2014 to 2021 from a general hospital in Vietnam, were resistant to almost all the antibiotics tested including erythromycin. The resistance of *S. aureus* to antibiotics may be as a result of the ability of the organism to produce β-lactamase enzyme which breaks down the β-lactam ring of the antibiotics and render them ineffective (Wolde *et al*., 2015). The resistance of the bacterial isolates to some of the antibiotics could be due to chromosomally or plasmid mediated resistant genes in the bacteria genetic make-up. This is associated with indiscriminate usage of antibiotics (Manisha *et al*., 2011).

Table 2: Antibiotic Susceptibility Profile of Bacterial Isolates from Hospital Kitchen Surface

Gram +ve	**PEF**	**CN**	**AM**	**CPX**	**S**	**SXT**	**APX**	**Z**	**R**	**E**	**MAR**
B. cereus	S	S	R	S	I	R	R	I	R	S	0.4
S. aureus	S	R	S	R	R	R	R	R	S	S	0.6
S. epidermidis	R	R	S	S	S	S	R	S	R	S	0.4
Micrococcus	S	S	S	R	S	S	R	S	R	S	0.3
Gram –ve	**PEF**	**CN**	**AM**	**CPX**	**S**	**SXT**	**AU**	**OFX**	**CH**	**SP**	
E. coli	R	R	R	S	R	S	R	S	S	R	0.6
P. aeruginosa	R	R	R	R	S	S	R	S	S	R	0.6
Enterobacter	R	R	R	S	R	S	R	S	S	R	0.5
K. pneumoniae	R	S	R	S	R	S	R	S	I	S	0.4

PEF: Pefloxacin, CN: Gentamycin, APX= Ampiclox, Z= Zinnacef, CPX= Ciprofloxacin, S= Streptomycin, SXT= Cotrimoxazole, E= Erythromycin, AM = Amoxicillin, R = Rocephin, CH = Chloramphenicol, SP = Sparfloxacin, AU = Augmentin, OFX = Ofloxacin

S = Sensitive, R = Resistance, I = Intermediate and MAR = Multiple Antibiotic Resistance Index; MAR index > 0.2 (Public health significance).

In this study, *P. aeruginosa* and *E. coli* exhibited high resistances to the antibiotics. Researcher had earlier reported the isolation of multiple antibiotic resistant *E. coli* from food handlers hand in schools in Selangor, Malaysia (Tan *et al*., 2014); from medical equipment and surfaces in children's emergency room from a Nigerian hospital (Ndu *et al*., 2019); and from food contact surfaces of a commercial kitchen in Kerala, India (Kunhiraman *et al*., 2023). Then Isichei-Ukah and Enabulele (2018) had earlier reported multidrug resistant *Pseudomonas aeruginosa* from Benin City, Nigeria. *Pseudomonas aeruginosa* exhibits resistance to a variety of antibiotics. Generally, the major mechanisms of *P. aeruginosa* used to counter antibiotic attack can be intrinsic, acquired or adaptive. The intrinsic resistance includes low outer membrane permeability, expression of efflux pumps that propel antibiotics out of the cell and the production of antibiotic-inactivating enzymes (Pang *et al*., 2019).

The multiple antibiotic resistances (MAR) indices of the isolates ranged from 0.3 to 0.6. *Staphylococcus aureus*, *E. coli* and *P. aeruginosa* had the highest MAR index of 0.6. It is frequently believed that MAR indices higher than 0.2 indicates high risk source of contamination and it's of public health concern (Krumperman, 1983). The high MAR indices of *S. aureus* and *P. aeruginosa* agreed with the works of Shayeghi *et al*. (2020) who reported same and concluded that the efficacy of many antibiotics for the treatment of severe infections had become limited due to the development of resistances.

Plasmid Analysis of Bacterial Isolates: Plate 1 shows the agarose gel electrophoretic separation of plasmid profile DNAs of isolates before curing. The detectable plasmid profiles in the bacterial isolates with band size ranged from 100 to 1000 bp. *Micrococcus* spp., *B. cereus, S. epidermidis* and *K. pneumoniae* harboured single plasmids while *P. aeruginosa*, *S. aureus, E. coli* and *Enterobacter* spp. harboured multiple plasmids with different sizes (Table 3). The plasmid profiles of the bacterial isolates from hospital kitchen surfaces before curing revealed that most of the tested isolates showed multiple antibiotic resistance to the tested antibiotics. Bacterial antibiotics resistance patterns are sometimes associated with the presence of large plasmids and ability of plasmids for conjugation process (Opere *et al*., 2013).

Plasmid Curing and Antibiotic Susceptibility Testing: After curing, the antibiotic susceptibility tests of the isolates showed that all the isolates, except *S. aureus*, *E. coli* and *P. aeruginosa* were sensitive to the antibiotics tested. *Staphylococcus aureus* was resistant to two antibiotics (gentamycin and ciprofloxacin), while *E. coli* and *P. aeruginosa* were resistant to gentamycin. The MAR indices were 0.2 and 0.1 respectively (Table 4).

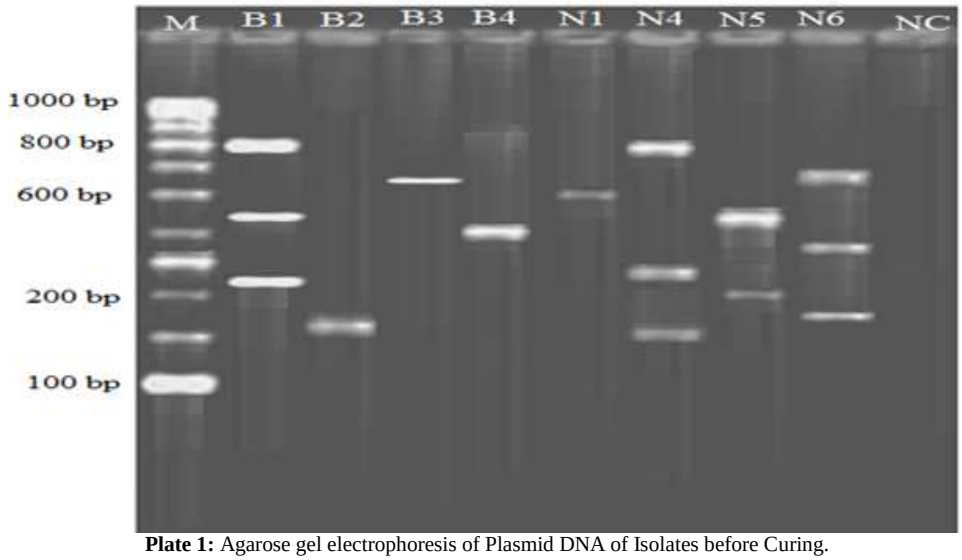

Plate 1: Agarose gel electrophoresis of Plasmid DNA of Isolates before Curing.

Key: Lane M-Molecular weight marker (1000 bp); Lane B1- *Staphylococcus aureus*; lane B2-*Micrococcus* spp.; Lane B3- *Bacillus subtilis*; Lane B4- *S. epidermidis*; Lane N1- *Klebsiella pneumoniae;* Lane N4- *Pseudomonas aeruginosa;* Lane N5- *Escherichia coli*; Lane N6- *Enterobacter* spp; Lane NC- Negative control (Distilled sterilized water).

Table 3: Plasmid profile of Bacterial Isolates before Curing

Isolate code	Bacterial Isolates	No. of plasmids	Sizes (bp)
B1	*Staphylococcus aureus*	3	800, 500, 250
B2	*Micrococcus* spp.	1	170
B3	*Bacillus subtilis*	1	680
B4	*Staphylococcus epidermidis*	1	400
N1	*Klebsiella pneumoniae*	1	600
N4	*Pseudomonas aeruginosa*	3	800, 300, 150
N5	*Escherichia coli*	2	500, 200
N6	*Enterobacter* spp.	3	700, 350, 180

Key: B1- *Staphylococcus aureus*; B2-*Micrococcus* spp.; B3- *Bacillus subtilis*; B4- *Staphylococcus epidermidis*; N1- *Klebsiella pneumoniae;* N4- *Pseudomonas aeruginosa;* N5- *Escherichia coli*; N6- *Enterobacter* spp.

Table 4: Antibiotic Susceptibility Profile of the Bacterial Isolates after Curing

Gram +ve	**PEF**	**CN**	**AM**	**CPX**	**S**	**SXT**	**APX**	**Z**	**R**	**E**	**MAR**
B. cereus	S	S	S	S	S	S	S	S	S	S	0.0
S. aureus	S	R	S	R	S	S	S	S	S	S	0.2
S. epidermidis	S	S	S	S	S	S	S	S	S	S	0.0
Micrococcus	S	S	S	S	S	S	S	S	S	S	0.0
Gram –ve	**PEF**	**CN**	**AM**	**CPX**	**S**	**SXT**	**AU**	**OFX**	**CH**	**SP**	
E. coli	S	S	S	S	S	S	S	S	S	S	0.0
P. aeruginosa	S	R	S	S	S	S	S	S	S	S	0.1
Enterobacter	S	R	S	S	S	S	S	S	S	S	0.1
K. pneumoniae	S	S	S	S	S	S	S	S	S	S	0.0

PEF: Pefloxacin, CN: Gentamycin, APX= Ampiclox, Z= Zinnacef, CPX= Ciprofloxacin, S= Streptomycin, SXT= Cotrimoxazole, E= Erythromycin, AM = Amoxicillin, R = Rocephin, CH = Chloramphenicol, SP = Sparfloxacin, AU = Augmentin, OFX = Ofloxacin S = Sensitive, R = Resistance, I = Intermediate and MAR = Multiple Antibiotic Resistance index. MAR index > 0.2 (Public health significance)

The results from Table 4 revealed that the isolates were sensitive almost all the antibiotics. Therefore, the antibiotic resistance initially exhibited by the isolates were plasmid mediated. Plasmids are small extra-chromosomal DNA molecules that carry genes that code for antibiotic resistance, virulence factors and activities in bacterial cells.

Conclusion: This study revealed that the hospital equipment surfaces were contaminated with highly resistant potential pathogens. All the isolates harboured plasmids with single and multiple bands. Resistance to antibiotics were encoded in the plasmid as most of the isolates became sensitive to the antibiotics after curing. Therefore, standard sanitation operation procedure and good hygienic practices

should be strictly observed in hospital kitchens to prevent food contamination and spread of infectious pathogens to patients.

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