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Gram-Negative Bacteria Associated with Laboratory Workbenches in Microbiology Department, University of Benin

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Abstract

More often than not, uncertainties in microbiological diagnosis could arise from the contamination of cultures in the laboratory. Contaminants in the lab could stern from the personnel, air currents, dusts, mites or other environmental components. This study was carried out to evaluate Gram-negative bacteria associated with laboratory workbenches in Microbiology Department, University of Benin, Benin City. Ten (10) workbenches were mapped out and evaluated for bacterial contamination before and after disaffection with 1% sodium hypochlorite solution (v/v). The bacterial isolates were enumerated and identified using standard culture-based techniques. Phenotypic virulence was evaluated for the bacterial isolates and Kirby–Bauer disc diffusion method was used for antibiotic susceptibility testing. The result revealed that disinfected or cleansed workbench surfaces had lower bacterial load compared to uncleansed surfaces. The disinfection could reduce bacterial contamination in the range of 55% - 92% if workbench surfaces are disinfected prior to analysis. The isolated bacteria identified in this study were *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella enterica. E. coli* (28.00%) was the most common contaminants in laboratory work benches while *E. aerogenes* (16.00%) had the least occurrence. *S. enterica* and *E. aerogenes* were recovered from laboratory workbenches even after disinfection with 1% hypochlorite solution. The bacterial isolates were found to harbour certain enzymes and factors, which contributes to virulence and by extension the public health significance of the isolates. The multiple antibiotic resistance index of the isolates, were above the permissible limit of 0.2 and thus were of public health importance. This study revealed that disinfection or cleaning of laboratory benches can reduce bacteria contamination from 55% to 92% using 1% sodium hypochlorite solution (v/v).

1. Introduction

The ubiquitous nature of microbes transcends time and location, and there is no safe haven for anyone who thinks he is safe. In the laboratory, microbes can be found in gears and instruments and even in high contact surfaces [1, 2, 3]. Contamination is defined as the presence of a small and undesired element (contaminant) in a natural environment, material, work environment and physical body amongst others [4]. Laboratory results can be negatively impacted due to the effect of bacterial contamination of cultures, which could arise from materials or environment [5, 6]. Microorganisms that are isolated from the laboratory environments can be referred to as environmental contaminants due to their unwanted presence in the laboratory environment and the adverse effects they may cause. Therefore, the avoidance or prevention of bacterial contaminants in the laboratory is

important for effective studies [7]. Bacteria begin to multiply after they have attached themselves to surfaces and diverse assemblages of organisms (biofilms) emerge, which, in combination with a wide range of particles, result in the complex structure of natural sediments within a short frame of time [8]. For the fact that microbes are ubiquitous, their presence in the lab cannot be eradicated completely, but can be controlled to decrease both their frequency and negative effects that may arise if dangerous variants are present [9]. One approach to controlling contamination in Microbiology laboratory is to employ aseptic technique. Aseptic methods help to achieve two essential goals which include prevention of microbial contamination of the laboratory environment as well as contamination with organisms from the environment [10]. The aforementioned can be accomplished by employing manipulation techniques that reduce the risk of generating aerosols and maintaining a clean and tidy laboratory [11]. Some containment levels are suited for laboratory-scale facilities, such as diagnostic, educational, research, clinical, and manufacturing facilities; these containment levels are sometimes referred to as "biosafety levels" [12]. Isolation of microorganisms from laboratory work spaces is carried out to assess the quality control of the lab, as well as check for the presence of harmful microorganisms, which might also contaminate microbial cultures. The presence of these pathogenic bacteria as environmental contaminants in Microbiology laboratories not only causes diagnostic difficulties, but also puts microbiologists, students, and other laboratory scientists at risk [13, 14]. Therefore, in order to prevent the contamination of microbiological culture, it became necessary to carry out this present study on evaluation of bacterial associated with laboratory workbenches in the Department of Microbiology, University of Benin, Benin City, Nigeria.

2. Methodology

2.1 Study design

The study was carried out at the Microbiology Laboratory of the University of Benin (UNIBEN), Benin City, from 2nd of June 2020 to 10th of July 2020. At the time, only a handful of students were on ground to carry out their project practical work in the Department of Microbiology. Specific areas (length x breadth) of the workbenches were marked out for sampling and prior to collection of samples, the dimensions of sampling areas were evaluated and a comparison between clean workbenches with 1% sodium hypochlorite (v/v) and unclean workbenches without any prior form of cleansing/disinfection was made. Quantitative approach to bacterial enumeration of workbench surfaces were evaluated.

2.2 Collection of Samples

Samples were collected from 10 laboratory workbenches with the aid of a sterile swab stick. From the Laboratory Work Benches, a total of 20 samples were taken, 10 of the samples were collected before the surfaces of laboratory work benches were cleaned and sterilized with 1% sodium hypochlorite (v/v) while the remaining 10 samples were collected afterwards. Samples collected before and after cleaning, were labeled appropriately. Immediately after sample collection, the swab was placed in a sterile nutrient broth from whence culture was done. The Bacterial colonies were subcultured in the appropriate growth medium. Pure cultures of the isolated bacteria were subcultured and incubated at 37 ^{o}C. Cultural, morphological and biochemical characteristics of the bacterial isolates were carried out using biochemical tests such as catalase, oxidase, indole, citrate, urease, triple sugar iron agar test, and urease test amongst others.

2.4 Enumeration of bacterial load from workbench surfaces

The method of choice for examination of workbench surfaces is via swabbing of a known area using a sterile swab that has been moistened in sterile saline (0.85%). This semi-quantitative approach enables enumeration of microorganisms per cm^2 and can facilitate interpretation of the results according to the method delineated by National Infection Service [15]. Sterile swab sticks,

aseptically moistened were used to swab a measured dimension of the surfaces of the workbenches. The swab sticks were then immediately transferred into 10 ml of tryptone soya broth. This according to NIS [15] is equivalent to 10^0 and gives a lower limit of detection of 10 colony forming unit (cfu) per swab if 1mL is plated. Further 10-fold serial dilution was then made to ascertain the total heterotrophic bacterial count using tryptone soya agar (Oxoid). The poured plates were incubated for 24 h. at a temperature of 28±2 ^oC. Total viable count was used to estimate the heterotrophic viable count for the samples in colony forming units per ml (cfu/m^2). Prior to collection a measured dimension of the surfaces was taken using a meter rule and the area of surfaces to be swabbed were aseptically marked and noted. Equation (1) below delineated by NIS [15] was used to estimate the number of viable bacteria isolates.

$$Bacterial\ count\ \left(\frac{cfu}{cm^2}\right) = \left(\frac{C}{V(n_1 + 0.1\,n_2)d}\right) x\ n_3 \qquad (1)$$

Where:
C = sum of colonies on the plates counted
V = volume of inoculum
n_1 = number of plates counted at first dilution
n_2 = number of plates counted at second dilution
n_3 = original volume of neat suspension
d = dilution from which the first count was obtained
The plates were incubated and colonies were counted using a colony counter. Colony forming units were computed and recorded. The value obtained is the count per swab and to calculate the count per cm^2, it was therefore divided by the swabbed area (cm^2). As previously described, the bacterial isolates obtained from the pour plates were identified using standard cultural and biochemical methods delineated by Ogofure et al. [2} and Bridson [16].

2.5 Determination of Phenotypic Virulence
Virulence factors, such as ability to degrade DNA, produce gelatinase, hemolysin, urease and lipase were carried out on the bacterial isolates. A 24-hour bacterial cell was standardized ($1.5x10^8$ cells/ml) and spread on DNAse agar plates (for DNAse activity). 5% sheep blood agar plates were used to culture isolated bacteria for their ability to produce hemolysin [17, 18].

2.6 Antibiotic Susceptibility Testing
Antibiotic susceptibility testing was performed on bacterial isolates using Kirby-Bauer Disc diffusion method. The following antibiotics (codes and concentration) used in the study include: MEM -Meropenem (10 µg), ERY - Erythromycin (15 µg), MET- Metronidazole (50 µg), AMC-Amoxicillin/clavulanic acid (20/10 µg), CL-Clindamycin (20 µg), CN-Gentamicin (10 µg), CIP-Ciprofloxacin (5 µg), and TET-Tetracycline (10 µg). Briefly, an 18 h. culture in broth was standardized using 0.5 McFarland scale and streaked on Mueller Hinton agar plates. With the aid of a sterile forceps, the discs were impregnated into the cultured plates. The medium was incubated for 24 h. at 37 °C. The plate was examined after incubation for zones of inhibition around each of the antibiotics. After then, the diameter of the inhibitory zone was measured in millimeters (mm) [19].

2.7 Multiple antibiotic resistance (MAR) index

In this study, the MAR index was determined by employing the methods delineated by Chitanand *et al*. [20]. Where the MAR index was obtained using the formula:

$$MAR\ index = \frac{y}{nx} \qquad (2)$$

$where\ y = number\ of\ resistance\ scored,$

$n = number\ of\ isolates\ and$

$x = total\ number\ of\ antibioitics$

According to Davis and Brown [21], an index of ≥ 0.2 and above is indicative of a 'high-risk' contamination source.

2.8 Statistical analysis

Before reporting the results achieved in this study, all data were subjected to statistical interpretation. The test utilized a probability level of 95 % for statistical significance ($p < 0.05$), and the data from the various parameters were statistically analyzed using IBM SPSS Statistics for Windows, Version 26.0.

3.0 Results and Discussion

The total heterotrophic bacterial count (Log_{10} cfu/cm^2) obtained from laboratory benches before cleaning ranged from 3.79±0.15 – 4.38±0.23 while bacterial counts after cleaning ranged from 3.30±0.09 – 3.47±0.12 (Figure 1) following treatment with 1 % sodium hypochlorite solution (v/v). More so, it was revealed that the bacteria burden on the laboratory workbenches before cleaning with disinfectant were higher when compared to the same benches after cleaning with disinfectant. There was a considerable significant difference in the bacterial load after cleaning workbenches (figure 1). The importance of disinfection of workbench surfaces in the laboratory cannot be overemphasized as it was revealed (Figure 2) that disinfection can remove or reduce approximately 55% - 92% of bacterial burden on work bench surfaces. The isolated bacteria identified in the study (Table 1) include *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus mirabilis* and *Salmonella enterica.* It is worthy of note that some of the bacteria were found in both cleansed and unclean workbenches. The bacterial isolates present in the laboratory were found to harbour certain enzymes and factors, which contributes to virulence and by extension the public health significance of the isolates.

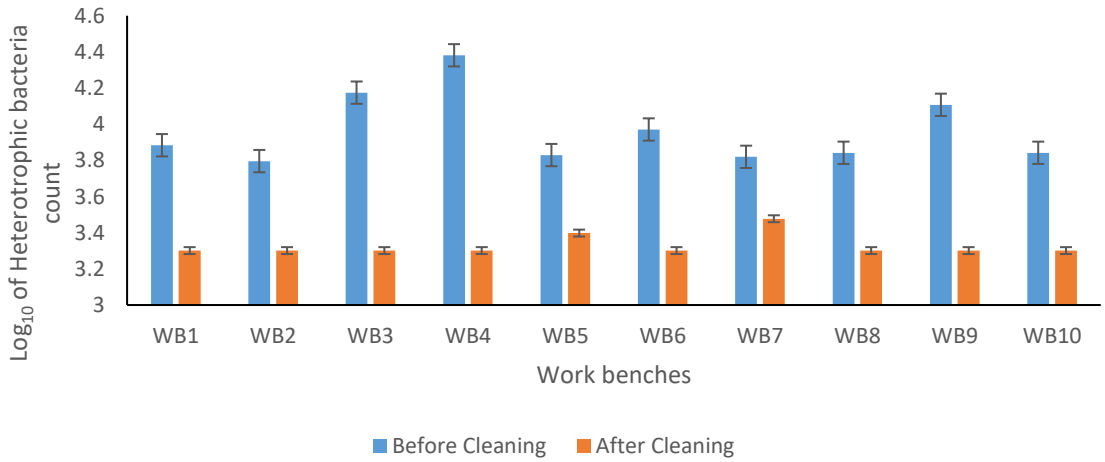

Figure 1. Heterotrophic bacterial count (Log_{10} cfu/cm^2) of laboratory workbenches in Microbiology department before and after cleaning

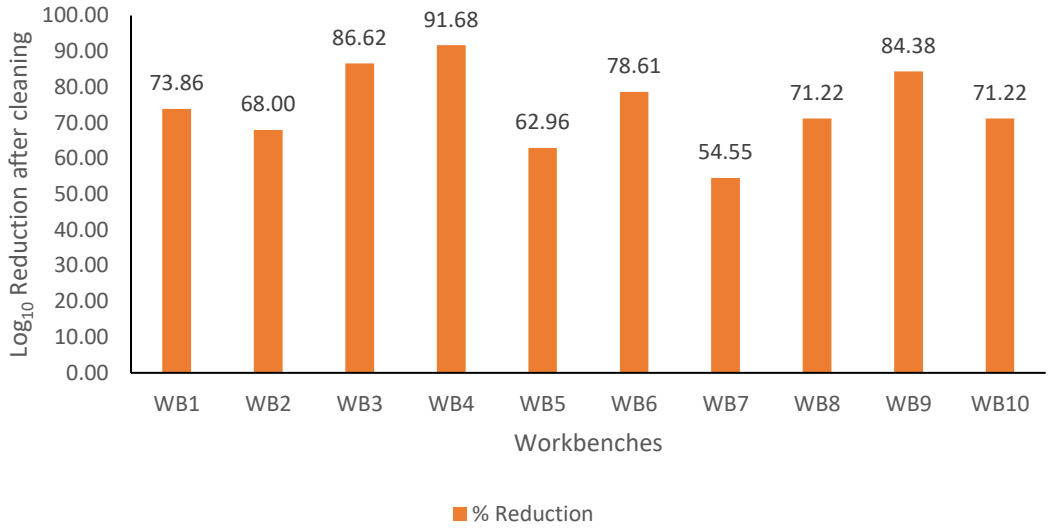

Figure 2. Bacterial log and percentage reduction after cleaning workbenches with hypochlorite solution

Table 1. Bacterial frequency of occurrence and distribution in laboratory workbenches

Isolates	Frequency (%)	Before cleaning	After cleaning
Escherichia coli	28.00	+	-
Salmonella enterica	16.00	+	+
Proteus mirabilis	20.00	+	-
Pseudomonas aeruginosa	20.00	+	-
Enterobacter aerogenes	16.00	+	+
Total	100.00		

Key: + present; - = absent

Table 2. Phenotypic virulence determinants of bacterial isolates

Isolates	Hemolysin	DNase	Gelatinase	Lipase	Urease
Escherichia coli	+	+	-	+	-
Salmonella enterica	-	+	+	+	-
Proteus mirabilis	-	+	-	+	+
Pseudomonas aeruginosa	-	+	-	+	+
Enterobacter aerogenes	-	+	+	+	+

Key: + present; - = absent

Table 3. Antibiotic susceptibility (%) pattern of the bacterial isolates from laboratory work benches

Isolates (n)	MEM	ERY	MET	AMC	CL	CN	CIP	TET
E. coli (7)	100	57	42	86	42	42	86	29
S. enterica (4)	100	75	50	75	50	75	75	25
P. mirabilis (5)	100	40	40	80	60	80	80	40
P. aeruginosa (5)	100	60	60	80	20	60	80	20
E. aerogenes (4)	100	25	50	75	50	75	75	25

Key: MEM -Meropenem (10 µg), ERY - Erythromycin (15 µg), MET- Metronidazole (50 µg), AMC-Amoxicillin/clavulanic acid (20/10 µg), CL-Clindamycin (20 µg), CN-Gentamicin (10 µg), CIP-Ciprofloxacin (5 µg), and TET-Tetracycline (10 µg).

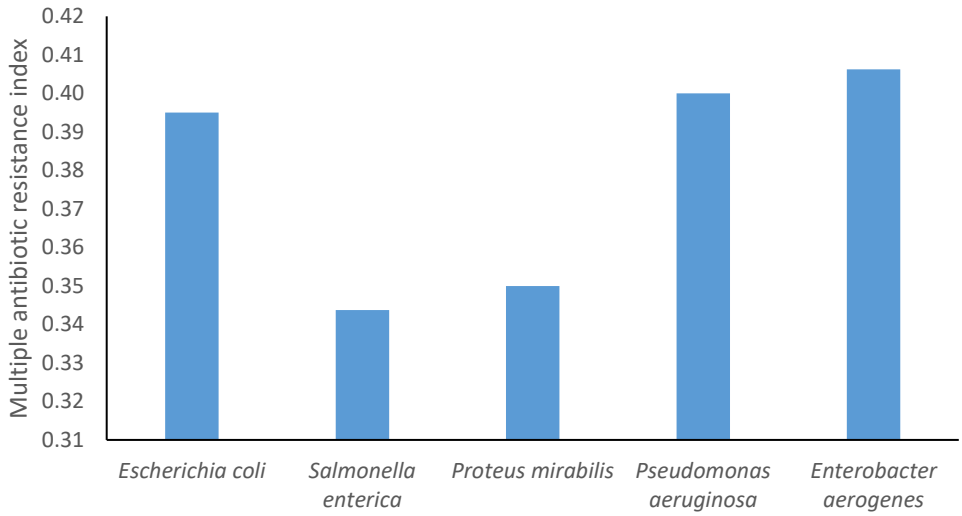

Figure 3. Multiple antibiotic resistance index of the bacterial isolates

The study focused on isolation and identification of bacterial isolates from laboratory workbenches. The result has revealed in this study was comparable to reports of Tuladhar et al. [22] who evaluated bacterial and viral contamination of surfaces after cleaning and disinfection. They opined that a 1 log reduction (90% reduction) was observed when surfaces was disinfected with 250 ppm chlorine (which is the active ingredient in sodium hypochlorite used in this study). Compared to benches without disinfection, there was higher bacterial contamination of workbenches by pathogenic Gram-negative. There was a considerable difference in the bacterial load after benches were cleansed with 1% sodium hypochlorite solution showing that bacterial loads on laboratory work benches could be reduced disinfection or cleaning of surfaces with sodium hypochlorite. The significant heterotrophic bacterial load present on workbenches in this study was consistent with the report of Mendoza *et al*. [9] and Tamburini *et al*. [23], who found a high load of Gram-negative bacteria on laboratory workbenches. They opined that, bags, cell phones, and computers brought in by laboratory workers and students might be a source of contamination. The bacteria isolated in this study include *E. coli*, *Salmonella enterica, Proteus mirabilis, Pseudomonas aeruginosa* and *Enterobacter aerogenes*. The isolation of *E. aerogenes, E. coli, P. mirabilis,* and *S. enterica*, demonstrates that monitoring contamination of workbench is critical, not only for the reliability of tests and experiments, but also for the safety of laboratory operators and casual visitors. Abatenh *et al.* [24] in consonance with results obtained in this, opined that unexpected data might arise from cross and self-contamination, as well as ongoing communication among doctors, laboratory personnel, and researchers in the laboratory. In the prevalence of the isolates from these laboratory samples, high average prevalence of 28% was observed to be *E. coli*. This could only indicate the persistence of the bacterium on workbenches. A few of the isolated bacteria were also recovered from the surfaces of work benches even after disinfection. This could also be a function of some bacteria to persist in the environment and resist the action of disinfectants to get rid of them completely. The prevalence of Gram-negative bacteria obtained in this study was in agreement with the report from studies carried out by Abatenh *et al.* [24]. The result of the antibiotic test against these isolates revealed a generally high resistance, characterized by average MAR index of 0.41. This high MAR index reflects a potential public health threat should a disease be established by this cause. Also, Chitanand *et al*. [17] described that the MAR index highlights the pathogens' importance in health threat and also its origin and exposure to antibiotics. Although, the sources of these pathogens may not be fully ascertained, with a few references to cross contamination and self-contamination of the sample culture, these contaminants are more likely of direct biological origin or with biological activities diffused in the air. This can

be further justified by the isolation of *Pseudomonas aeruginosa* which has been earlier reported as being associated with airborne contamination. However, the study by Tamburini *et al.* [19] has described that surface characteristics (such as roughness) greatly influence surface susceptibility to microbial deposition, even as unexpected load was obtained on stainless surfaces, which was attributed to electrostatic forces of attraction.

4.0 Conclusion

This study revealed that disinfection or cleaning of laboratory benches can reduce bacteria contamination from 55% to 92% using 1% sodium hypochlorite solution (v/v). It does also reveal that lab benches are contaminated with bacteria isolates of public health significance thus the usefulness of cleaning or disinfection cannot be overemphasized.

References

[1] C.R. Arias, K. Koenders and Larsen, A.M. (2013). Predominant bacteria associated with red snapper from the Northern Gulf of Mexico. Journal of Aquatic Animal Health vol. 25 pp 281–289.

[2] A.G. Ogofure, I.O. Bello-Osagie, C.O. Ahonsi, B.U. Aduba and A.O. Emoghene (2018). Qualitative detection and isolation of bacteria from surfaces of canned drinks sold in Ugbor, Benin City. Annals of Science and Technology vol. 3(2) pp 20-25.

[3] A.G. Ogofure, J.Z. Saidu, P.G. Olannye, C.O. Ahonsi and A.O. Emoghene (2019). Qualitative detection of methicillin resistant *Staphylococcus aureus* from the surfaces of canned drinks sold in University of Benin campus. Nigerian Research Journal of Engineering and Environmental Management vol. 4(1) pp 227-235

[4] S.M. Collins, D.M. Hacek, L.A. Degen, M.O. Wright, G.A. Noskin and L.R. Peterson (2001). Contamination of the clinical microbiology laboratory with vancomycin-resistant enterococci and multidrug-resistant Enterobacteriaceae. Journal of Clinical Microbiology vol. 39 pp 3772–3774.

[5] H. Jung, W. Shih-Yee, Y. I-Wen, H. Ding-Wei, Y. Wei-Ju, W. Tzu-Hao and W. Hsin-Shih (2003). Detection and treatment of *Mycoplasma* contamination in cultured cells. Chang Gung Medical Journal Vol. 26(4) pp 250-258.

[6] A.Z. Mahmoudabadi (2007). Laboratory instrument contamination with dermatophytes–a risk for dermatophytosis. Letters in Applied Microbiology Vol. 44 pp 112–113.

[7] A. Okuniola, A. Adewoyin and A. Oluwatoyin (2007). Evaluation of pharmaceutical and microbial qualities of some herbal medicinal products in South Western Nigeria. Tropical Journal of Pharmaceutical Research Vol. 6(1) pp 661-670.

[8] R.M. Donlan (2002). Microbial Life on Surfaces. Journal of Emerging Infectious Diseases Vol. 8(9) pp16-21

[9] J. Mendoza, W. Caso, C. Valdez, M. Pons, L. Valle, V. Ore, D. Michelena, J. Mayra, V. Gavidea, M. Vargas and J. Ruiz (2014). Diagnosis of carrion's disease by direct blood PCR in thin blood smear negative samples. Journal of Clinical Microbiology Vol. 9 pp 92-183.

[10] M. Ghayoor, A. Qadoos, S. Bahadar, A. Hayat, M. Daud, A. Hassan, F. Ali, A. Zeb, K. Rahman, A. Wahab and Z. Fatima (2015). Isolation and Identification of Common Contaminants Bacteria from Working Area in Microbiology Laboratory. Journal of Bio-Molecular Sciences (JBMS) vol. 3(2) pp 74-78.

[11] A. Knox (2010). Guidelines for cleaning, disinfection and sterilization of patient care equipment. Infection control committee. NHS Dumfries and Galloway Board-wide vol. 1: pp 54.

[12] C. Cheng, J. Sun, F. Zheng, K. Wu and Y. Rui (2014). Molecular identification of clinical "difficult-to-identify" microbes from sequencing 16S ribosomal DNA and internal transcribed spacer 2. Journal of Clinical Microbiology Vol. 13 pp 1-7.

[13] F. S. Rhame (1998). The inanimate environment. In**:** *Hospital infections*. J.V. Bennett and P.S. Brachmann (Eds.). Philadelphia, Lippincott-Raven pp 299-324.

[14] J. Konar and S. Das (2013). Common contaminants of bacteriology laboratory: Microbiological Paramores. *International journal of pharmaceutical science invention* **2**(11):36-37.

[15] National Infection Service (2017). *Detection and Enumeration of Bacteria in Swabs and Other Environmental Samples* (Vol. 4). Public Health England 22pp.

[16] E.Y Bridson (2006). *The Oxoid Manual* (9th Edition). Oxoid Limited, Basingstoke Hamsphire, England 623 pp.

[17] E.O. Igbinosa (2016). Detection and antimicrobial resistance of *Vibrio* isolates in aquaculture environments for public health. *Microbial Drug Resistance***22**(3): 238 - 245.

[18] E. Igbinosa, A. Beshiru, I. Igbinosa, A. Ogofure, and K.E. Uwhubia (2021). Prevalence and characterization of food-borne *Vibriopara haemolyticus* from African salad in Southern Nigeria. *Frontiers in Microbiology.*

[19] D. Brown and A. Macgowan (2010). Harmonization of antimicrobial susceptibility testing breakpoints in Europe: implications for reporting intermediate susceptibility. Journal of Antimicrobial and Chemotherapy, Vol. 65 pp 183–185.

[20] M. Chitanand, T. Kadam, G. Gyananath, N. Totewad and D. Balhal (2010). Multiple antibiotic resistance indexing of coliforms to identify high-risk contamination sites in aquatic environment. Indian Journal of Microbiology Vol. 50 pp 216–220.

[21] R. Davis and P.D. Brown (2016). Multiple antibiotic resistance index, fitness and virulence potential in respiratory *Pseudomonas aeruginosa* from Jamaica. Journal of Medical Microbiology Vol. 65 pp 261–271.

[22] E. Tuladhar, W.C. Marion, M.H. Koopmans, R. Zwietering, R. Beumer, and E. Duizer (2012). Residual viral and bacterial contamination of surfaces after cleaning and disinfection. Applied and Environmental Microbiology Vol. 78: 7769 –7775

[23] E. Tamburini, V. Donegà, M.G. Marchetti, P. Pedrini, C. Monticelli and A. Balbo (2015). Study on Microbial Deposition and Contamination onto Six Surfaces Commonly used in Chemical and Microbiological Laboratories. International Journal of Environmental Research and Public Health Vol. 12 pp 8295-8311.

[24] E. Abatenh, B. Gizaw and Z. Tsegaye (2018). Contamination in a Microbiological Laboratory. International Journal of Research Studies in Biosciences Vol. 6(4):7-13.