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Quantitative Detection of Methicillin Resistant *Staphylococcus aureus* from the Surfaces of Canned Drinks Sold in University of Benin Shopping Complexes

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ABSTRACT

This study was carried out to detect the presence methicillin resistant Staphylococcus aureus from the surfaces of canned drinks sold in University of Benin shopping complexes. Standard culture-based techniques were used to screen for MRSA from one hundred canned drinks samples. Total bacteria count was enumerated from the surfaces of canned drinks while staphylococci and MRSA were identified using mannitol salt agar and Oxacillin resistant screening agar base supplemented with ORSAB supplement (Oxoid) along with other tests. Hemolysin production was carried out on Columbia agar blood base (Oxoid). The antibiogram and multiple antibiotic resistance were evaluated using standard methods. The results obtained in this study shows that bacterial contamination of refrigerated canned drink surfaces was found to be 5%, 20% and 10% for basement, life sciences and physical sciences shopping complexes respectively. About 37.5% and 12.5 % of canned drink surfaces stored in the crates were found to be contaminated in Physical Sciences and June 12 shopping complex. The highest Staphylococcal count from the surface of canned drinks was found to be 1.15 x 10^3 cfu/cm^2. A few samples which had over 300 colonies (too numerous to count) in 50% of the shopping complexes evaluated in the study while there were also samples which were devoid of staphylococcal contamination. All isolated Staphylococcal species were found to be hemolytic and the MRSA strains were coagulase positive. The MRSA strains had higher antibiotic resistance index of 0.51 compared to 0.47 for other staphylococci and they are of public health importance.

1. INTRODUCTION

The surfaces of canned drinks have been muted as a carrier of microorganisms particularly bacteria which are ubiquitous in nature (Ogofure *et al.,* 2018). These bacteria have also been implicated as a cause of some

serious health challenges particularly for immunocompromised individuals with multiple resistance index of the isolated bacteria from canned drinks surfaces above the normal stipulated value of 0.2 (Ogofure *et al.,* 2018). There have been several reports on the contamination of surfaces of objects by bacteria ranging from computer keyboards; to automated teller machine keypads (Kapdi *et al.,* 2008; Famurewa and David, 2009; Onochie *et al.,* 2013; Kigigha and Jonathan, 2017; Akinnibosun and Adetitun, 2018). More so, there are several other studies which have shown the ubiquity of microorganisms in connection to public health in that they are able to colonize or contaminate other surfaces such as mobile phones of hospital staff (Kilic *et al.,* 2009), beverage packages (Dantas *et al.,* 2006) as well as food and household surfaces (Kusumaningrum *et al.*, 2002; Othman, 2015).

Pelczar *et al.* (1993) posited that bacteria have a reputation to survive in different environment that are depleted in nutrients and moisture such as clothing, glassware and other inanimate objects. It has often been said that the mere presence of certain pathogenic bacteria in a particular place at a certain time is not enough to pose the required threat to humans or public health. As equally important as their presence is their number (which could be their density or population) in the said environment (Prescott *et al.,* 2008). The quantitative analysis of bacteria from aforementioned inanimate objects exist for studies which have been cited earlier but there are not many studies which have tried to estimate the population of certain species of bacteria on the surface of canned drink samples. At best, Ogofure *et al.* (2018) qualitatively evaluated the bacterial contaminants on the surfaces of canned drinks in Ugbor, Benin City. There are also not many publications about the public health significance of bacterial contamination of canned drink surfaces and associated health risk to consumers especially in third world countries (Mustafa *et al.,* 2015; Ogofure *et al.,* 2018).

Staphylococcus aureus according to Cheesbrough (2000) is a bacterium that colonizes the human skin and nasal passage. It possesses two basic mechanisms to cause damage to humankind and they are via active tissue invasion through the building of abscesses as well as via the release of toxins that can kill cells. Methicillin – resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus*, which is resistant to methicillin and related antibiotics and is particularly difficult to treat because it is also resistant to other common antibiotics (Mansouri and Khaleghi, 1997). Although *Staphylococcus aureus* infections were historically treatable with common antibiotics, but the emergence of drug–resistant strains are now a major concern (Mansouri and Khaleghi, 1997). In this study, the aim was to isolate and characterize methicillin resistant *Staphylococcus aureus* from surfaces of canned drinks sold in University of Benin shopping complexes, Ugbowo campus, Benin City.

2. MATERIALS AND METHODS

2.1. Preparation and Collection of Samples

Prior to sampling and laboratory analysis, efforts were geared towards the propagation of aseptic procedures (preventing contamination during analysis). The materials used in the study were branded Oxoid analytical grade. The Oxoid manual (2006) was duly consulted in terms of the media for best recovery of the target bacteria (*Staphylococcus aureus* cum MRSA) of interest in this study (Bridson, 2006). The media were prepared according to manufacturer's specification and estimation of Staphylococcal population was made possible using the method delineated by public health England. The sample as earlier noted was aseptically collected using sterile Amies swab stick which was soaked with tryptic soy broth to make the tip more absorbent and increase the possibility of recovering bacterial contaminants on the surfaces of the canned drink samples.

2.2. Sampling Design

The University of Benin is a micro community where commercial and economic activities thrive with well over ten thousand students on campus. There are over twelve shopping complexes serving the myriads of

students and as such, the study was designed in such a way that 90% of the shopping complexes covered in the research. A total of hundred (100) samples were obtained from ten different complexes in University of Benin and analyzed for the presence of methicillin resistance *Staphylococcus aureus* and other possible staphylococci. More so, in a bid to create diversity, the samples stored in the refrigerator were taken into consideration since most students would prefer the drink served cold. Those stored in the crates before being placed in the refrigerator were also considered.

2.3. Enumeration and Isolation of Microorganisms

The method of choice for examination of surfaces is swabbing of a known area (10-100 cm^2) using a sterile swab that has been moistened in 5 ml of tryptone soya broth. This semi quantitative approach enables enumeration of the microorganisms per cm^2 and can facilitate interpretation of the results according to the method delineated by Public health England (2014).

Sterile swab sticks, aseptically soaked with 10 ml of tryptic soy broth were used to swab the upper surfaces of canned drinks which come in direct contact with the mouth. The swab sticks were then immediately aseptically transferred into 5 ml of tryptone soya broth. This according to Public health England (2014) is equivalent to 10^0 and gives a lower limit of detection of 10 cfu per swab if 1 mL is plated. Further 5-fold serial dilution was then made to ascertain the total heterotrophic bacterial count using Mueller Hinton agar (Oxoid) and total staphylococcal count was done using Mannitol salt agar. The total bacterial count on Mueller Hinton agar was used to estimate the total viable count for the samples in colony forming units per ml (cfu/m^3). Equation (1) was used to estimate the amount or density of bacteria from the surfaces of canned drinks.

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

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 value obtained is the count per swab to calculate the count per cm^2 it will therefore be divided by the swabbed area. In this case 78.57 cm^2 (the circular surface of the canned drink having a radius of 5 cm)

2.4. Isolation of *Staphylococcus aureus* and MRSA

The bacteria colonies appearing yellow on mannitol salt agar (MSA) where further screened using another selective/differential media known as the oxacillin screening agar base (ORSAB) supplemented with ORSAB selective supplement SR0195 (Oxoid). The growth of blue colonies on ORSAB having initially appeared as yellow on MSA were considered as MRSA. They were further characterized using biochemical means such as coagulase and catalase tests as well as the use of Gram stain delineated by Cheesbrough (2006). The ability of the isolated staphylococcal species to lyse the red blood cell was also deciphered on blood agar plates indicating the kind of damage it could cause when it gains entry into the blood. The hemolytic test as well as the coagulase test are a proof of the virulence of the isolated bacteria species.

2.4.1. Determination of hemolysin production

Hemolysin Production was detected using the method described by Drews *et al.* (2005). The bacteria isolates were cultured overnight at 37 ºC on Columbia agar blood base (Oxoid) plates, which was supplemented with 5% defibrinated sheep blood. The isolates, which were able to lyse red blood cell (hemolysis), were regarded as positive for hemolysis.

2.4.2. Standardization of bacterial isolates for antibacterial susceptibility

Following characterization and identification of the isolated bacteria, a standardized number of inoculum were prepared to carry out the antibiogram. McFarland Standard of 1.5 x 10^8 cells/ml was prepared and its turbidity was correlated using a defined solution of HCl and $BaCl_2$. The absorbance of the solution was determined using a spectrophotometer after which the definite estimate of bacterial cells used for antibiogram (CLSI, 2017).

2.4.3. Antibiotics susceptibility test

The guidelines stipulated by CLSI (2017) was strictly followed in the determination of susceptibility and resistance of the isolated Staphylococcal. The antibiogram of the isolates were determined by the disk diffusion method on Mueller-Hilton agar. The following antibiotics; Meropenem (10 µg), Gentamicin (30 µg), Vancomycin (30 µg), Amoxicillin (30 µg), Ciprofloxacin (5 µg), Sulphamethoxazole (25 µg), Ceftazidime (30 µg), Erythromycin (15 µg) were tested against the isolates and incubated at 37 ºC for 24 h. The bacteria in broth culture were used for this process. The turbid broth was then diluted to be equivalent to a 0.5 McFarland standard (1.5 x 10^8 cfu/ml). Swab sticks were used to spread the turbid inoculums containing the activated isolate on the nutrient agar plate. The disc diffusion method was used for susceptibility testing. The antibiotic discs were carefully and firmly placed on the inoculated Muller Hinton agar plates using a sterile pair of forceps. The diameter of the zone of inhibition was measured in millimeters (mm) using a meter rule (Igbinosa and Obuekwe, 2014). Incubation of the plates were done at 37 °C for 18-24 h and the results were taken as resistant, intermediate or sensitive, according to the standards stipulated by Clinical and Laboratory Standards Institute (2017).

2.4.4. Multiple antibiotic resistances (MAR) index

The MAR index is a good tool for health risk assessment, which identifies if the isolates are from a region of high or low antibiotic use Davis and Brown (2016). A MAR index of 0.2 and above indicates a 'high-risk' source of contamination. The multiple antibiotic resistance MAR index was determined for each isolate using the methods delineated by Chitanand *et al.* (2010) by dividing the percentage of antibiotic resistance of the entire antibiotics used in the study to the total possible number percentage of antibiotics used. It is however, an extension of the formula delineated by Krumpernam (1983), whose equation below tells the story.

$$MAR\ index = \frac{a}{b} \tag{2}$$

Where a is the number of resistant antibiotics used in the study and b is the total number of antibiotics used

2.4.5. Statistical analysis

Data were analysed using statistical package for social scientist (SPSS) version 21.0 and Microsoft Excel (2016). Descriptive statistics and percentages were used to make comparisons of most of the data obtained.

3. RESULTS AND DISCUSSION

In this study, one hundred (100) samples from different randomly selected shopping complexes in University of Benin was evaluated for the presence and prevalence of methicillin resistant *Staphylococcus aureus* (Table 1). While it is true that we live amongst myriads of microorganisms, Otu-Bassey *et al.* (2017) reported that it is inevitable that we live amongst millions of microorganisms as they are found in the air we breathe, the food we eat and on our body surfaces as well as other close environments (Otu-Bassey *et al.,* 2017). It is worthy of note that contamination of the surfaces of canned drinks can be possible from different points as most canned drink surfaces are apparently sterile after production from the factories even to the point where they are distributed to retailers and consumers (Ogofure *et al.,* 2018). However, most contamination could be because of environmental influences such as the air quality, personal hygiene of handlers, presence and quantity of aerosolized droplets in the storage environment and contamination from other sources. The results obtained in this study is similar to that obtained from Ogofure *et al.* (2018) who isolated *Staphylococcus aureus, Bacillus cereus* and *Pseudomonas aeruginosa* from surfaces of canned drink samples sold. Similar report was also obtained in the research of Dantas *et al.* (2006). Since most students prefer to consume canned drinks when cold, it became necessary to evaluate the contamination level of the samples in the refrigerator.

Table 1: Frequency of bacterial contaminants of refrigerated and non-refrigerated canned drinks sold in shopping complexes in University of Benin

Sample location (n)	Refrigerated (%)		Crates (%)	
	Contaminated	Non-contaminated	Contaminated	Non contaminated
Basement (20)	5	95	0	100
Life Science (20)	20	80	0	100
Physical science (18)	10	90	37.5	62.5
June 12 (17)	0	100	12.5	87.5
Faculty (04)	25	75	0	100
Twin LT (08)	0	100	0	100
Main gate (09)	0	100	0	100
Medical complex (04)	0	100	0	100

Table 2: Total staphylococcal count of canned drink surfaces in University of Benin

Sample location (n)	(Mean ± S.E)10^3			
	Lowest	Highest	Samples with no growth (n)	Samples with too numerous to count (n)
Basement (20)	0.01	0.72	09	00
Life Science (20)	0.04	1.15	04	01
Physical science (18)	0.03	0.17	08	02
June 12 (17)	0.01	0.70	5	2
Faculty (04)	0.00	0.08	01	00
Twin LT (08)	0.00	0.18	06	00
Main gate (09)	0.10	6.70	05	00
Medical complex (04)	0.01	0.82	00	01

Most consumers of soft drinks usually prefer it to be served cold and thus would want to purchase refrigerated drinks. Bacterial or staphylococci contamination of refrigerated canned drink surfaces was found to be 5%, 20% and 10% for basement, life sciences and physical sciences shopping complexes respectively (Table 1). The levels of contamination observed in refrigerated samples are likely to be influenced by a range of factors including the nature and levels of initial contamination introduced on contaminated canned drinks surfaces and the hygiene of those placing drinks or other materials into the refrigerator as well as the efficiency and frequency of refrigerator maintenance and cleaning (FDA, 2015). Refrigeration is one of the most widely practiced methods usually intended for controlling microbial growth on perishable food products (FDA, 2015) of which temperature specification of four to five (4 -5°C) degree Celsius is considered desirable. Otu-Bassey *et al.* (2017) opined that these undesirable organisms may have entered the refrigerators from unwashed foods, unclean hands, fluctuation in temperature (especially during inconsistency in power supply), and unclean container surfaces introduced into the refrigerator. This was also similar to the report of Ogofure *et al.* (2018) where all samples analyzed from registered distributors showed no bacterial growth. Meanwhile, other contaminated samples were primarily from local vendors and from refrigerators.

Otu-Bassey *et al.* (2017) opined that the undesirable organisms, which in this case is *Staphylococcus aureus* might have entered the refrigerators from unwashed foods, unclean hands, through an opened refrigerator door, warm temperature, and unclean container surfaces introduced into the refrigerator. More so, it is very likely that unstable power supply also might contribute to the growth and proliferation of bacteria in the refrigerator. The economic reality of power failure is that most refrigerators can then be compared to microbial incubators in disguise. The presence of contaminating bacteria in the surfaces of canned drinks through either direct or cross contamination of other stored foods, may result in food borne illness if ingested.

The antibiotic susceptibility and resistance pattern of staphylococcal isolates obtained from the surfaces of canned drinks surfaces is shown Tables 3 and 4. The staphylococcal isolates were susceptible to gentamicin (100 %), Ceftazidime (55.55 %), ciprofloxacin (50.00 %) and Meropenem (55.55 %). While the MRSA isolates were found to be susceptible to gentamicin (75.00 %), Meropenem (50.00 %) and Ceftazidime (25.00 %). The susceptibility and resistance profile of the bacterial isolates is typical of the results obtained in the report of Ogofure *et al.* (2018) where the staphylococcal isolates were found to be resistant to Augmentin, erythromycin and a host or related antibiotics. The susceptibility or resistance pattern of the isolated staphylococci showed that the bacteria were multi resistant and as such are of public health importance in that they are resistant to more than two antibiotic classes used in the study (Table 5).

Table 3: Antibiotic susceptibility pattern of isolated bacteria

Isolates (n)	Antibiotic susceptibility of isolates (%)							
	CIP	MEM	CN	CAZ	VA	AMC	RL	E
MRSA (4)	(75)	(50)	(75)	(25)	(0)	(0)	(0)	(0)
S. aureus (9)	(50)	(55.5)	(100)	(55.5)	(22.2)	(0)	(0)	(0)

MEM=Meropenem (10 ug), CN = Gentamicin (10 ug), VA= Vancomicin (30 ug), AMC = Amoxycilin (30 ug), CIP= Ciprofloxacin (5 ug), RL= Sulphamethoxazole (25 ug), CAZ = Ceftrazidine (30 ug), E= Erythromicin (15 ug)

Table 4: Antibiotic sensitivity and resistance pattern of isolated bacteria

Isolates (n)	Antibiotic resistance pattern of isolates								Percentage (%)
MRSA (4)	CIP	MEM	CN	CAZ	VA	AMC	RL	E	47.5
S. aureus (9)	CIP	MEM	CN	CAZ	VA	AMC	RL	E	51.1

MEM=Meropenem (10 ug), CN = Gentamicin (10 ug), VA= Vancomicin (30 ug), AMC = Amoxycilin (30 ug), CIP= Ciprofloxacin (5 ug), RL= Sulphamethoxazole (25 ug), CAZ = Ceftrazidine (30 ug), E= Erythromicin (15 ug)

Figure 1 revealed that the isolated organisms were of public health importance and that they have been exposed one way or the other to antibiotics as depicted by their multiple antibiotic resistance. None of the isolated pathogens were within the safe range of 0.2. Insomuch that the MAR index is greater than 0.2 is more than enough reason to worry about the challenge to public health posed by these bacteria isolates from the surfaces of canned drinks. Consumers should be very careful and make conscious efforts to always rinse or wash the surface before drinking directly.

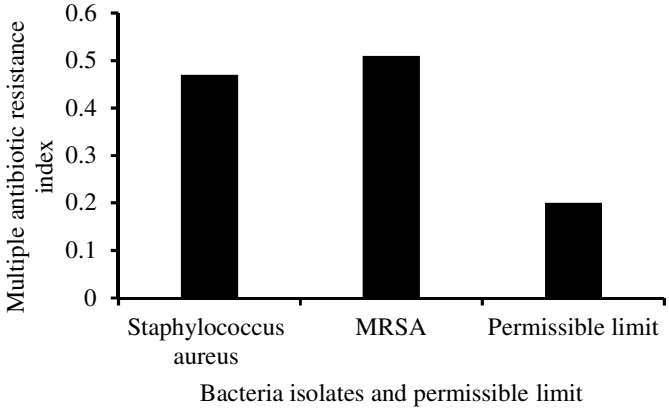

Figure 1: Multiple antibiotic resistance index (MAR) of isolated pathogenic *Staphylococci*

The presence of putative bacterial isolates such as *S. aureus,* a common inhabitant (up to 50%) of the human nose, throat, and skin is perhaps more likely to contaminate surfaces of canned drinks by direct or indirect human contact during handling and storage (Prescott *et al*., 2005; Willey *et al*., 2008). As a Gram-positive organism, it is relatively resistant to drying and is, therefore, more likely to become dominant than more desiccation-sensitive organisms, especially in the low water activity conditions which prevail in refrigerators and canned drink surfaces (Otu-Bassey *et al.,* 2017). The main reservoir of *S. aureus* is the hand from where it is introduced into food during handling and preparation (Hui *et al*., 2001). The MAR index according to Chitanand *et al.* (2010) reflects the pathogen's importance as a public health threat and more so, its origin (whether or not antibiotics have been used).

4. CONCLUSION

It is important to clean and sanitize any surface that is exposed to food and or other materials known to harbour a plethora of bacterial pathogens. Surfaces of canned drinks have been found to harbour multiple resistant pathogens of public health importance and the extent of cleaning the surfaces of canned drinks before consumption is paramount to reducing or removing contaminants from the surfaces of canned drinks.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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