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Multiple Antibiotics Resistance Index of Bacteria Isolated from Coolers used for the Sales of Soft Drinks

Jemikalajah D. Johnson
Department of Microbiology,
Faculty of Science,
Delta State University Abraka,
Delta State, Nigeria.
E-mail: jemikalajahjohnson2007@yahoo.com

Abstract
Bacteria contribute to high infection rate due to their colonisation and dissemination among humans and ability to developing resistance to antibiotics. This study was aimed at the determination of antibiotics resistance to bacteria recovered from coolers for sales of soft drink in Kwale, Delta State, Nigeria. One hundred samples from the inner surface of the coolers from different shops and location in Kwale metropolis were examined using standard bacteria culture methods. Antibiotic susceptibility testing was done using Kirby-Bauer modified disc diffusion technique. Results revealed that Staphylococcus aureus had incidence of 16 (53.3%), Escherichia coli 11(36.7%) and Salmonella spp. 3(10.0%). Also, Staphylococcus aureus was sensitive to 3 antibiotics, Escherichia coli 4 and Samonella spp 5. Multiple antibiotic resistance indices of 0.7, 0.6 and 0.5 were obtained for Staphylococcus aureus, Escherichia coli and Salmonella spp. respectively. This study has shown that bacteria identified were resistant to more than one antibiotics and confirm the presence of multiple antibiotic resistance bacteria in the community studied.

Keywords: Antibiotics, Bacteria, Cooler, Index, Resistance

INTRODUCTION
The adhesion and persistence of microorganisms to surface can spread pathogens to foods and affect their safety (Bae *et al.*,2012). Previous findings revealed the ability of microorganisms to attach to all the surfaces in the environment, such as food and wood (Siroli *et al.*, 2014). Additionally, if microorganisms remain on a given surface for a relatively long time, they can multiply and, eventually, form biofilms (Uhlich *et al.*, 2006). Although no reports on the isolation of microorganisms from storage facilities such as coolers has been recorded but studies have shown that various pathogens, including *Escherichia coli* and *Listeria monocytogenes*, have been recovered from utensils and equipment surfaces (Martinon *et al.*, 2012).

Microbial cross-contamination is the transfer of microorganisms from one item to another (Minnesota Department of Health, 2007). In food, cross contamination is a major concern

*Author for Correspondence

since it increases the risk of the intake of contaminated food. However, cross-contamination of pathogens from inert surfaces to foods has been documented (Erickson *et al.,* 2015).

Some bacteria resistance to antimicrobials are found in nature while others may acquire resistant genes from bacteria in the environment such as water or soil. The natural cause of bacteria resistance is mutation of R-plasmid exchange between bacteria of the same species (Bell *et al.,* 2010). The commonly use antibiotics in poultry or agricultural practices to prevent diseases can contaminate surface and underground water (El-Zanfaly *et al.,* 2018) from where it can be transferred to humans in coolers for sales of drinks thereby adding to the resistance problem. Also, the inappropriate use of antibiotics in preventing or treating human infections is believed to be the common cause of bacterial resistance. When these antibiotic resistant bacteria are introduced into water through faecal contamination, its emerging diseases will hardly respond to treatment (Walter and Vennes, 2015). It is common that bacteria that develop resistance to one antibiotic may also have the ability to develop resistance to another antibiotic which is usually referred to as multiple-antibiotic resistance (Walter and Vennes, 2015).

Aim of the study

This study was aimed at the determination of the multiple antibiotics resistance index of bacteria isolates of coolers in Kwale.

MATERIALS AND METHODS

Kwale community Delta State, Nigeria was located approximately between Longitude 5^{o}.00′ and 6^{o}.54′ East and latitude 5^{o}.00′ and 6^{o}.30′ North of Delta State with a population 114, 17 and about 71 kilometre north of Asaba (World gazetteer, 2007).It is a semi urban area with lively activities such trading, farming, learning to acquire entrepreneurship skill and oil exploration which attracts residents which creates room for social interactions. All of these can contribute to transmission of pathogens from the handlers of liquors to consumers and eventually contamination of cooling or storage facilities. This may ultimately result to infections of consumers and emergence of bacteria resistant strains.

Sample collection

One hundred swab samples were collected by random sampling techniques from coolers in different shops. This was done by swabbing the inner surface of the coolers and the swabs labelled appropriately. Samples were transferred to Microbiology Laboratory unit, Delta State University, Abraka for analysis.

Isolation of bacteria

Culture method was used for the isolation of bacteria. The swab samples were cultured on already prepared MacConkey, Blood and Chocolate and incubated aerobically at 37^{o}C for 24hrs. The bacterial isolates were identified by Gram staining, motility testing, catalase, coagulase, indole and other biochemical tests as described by Cheesbrough (2000)

Bacteria susceptibility testing

This was carried out using the Kirby Bauer modified disc diffusion technique. Multi antibiotic disc paper already impregnated with a known volume and appropriate concentration of antimicrobial agents was placed on a plate of Mueller Hinton agar already inoculated with the test organisms. The plates were incubated aerobically at 37^{o}C for 24hrs and the size of the zone of inhibition measured and compared with Clinical Laboratory Standard institute guidelines (2012).

Determination of multiple antibiotic resistance (MAR) index

This was determined for each tested bacterium by the formula MARI= a/b, where a is the number of antibiotics to which the organisms were resistant and b the total number of antibiotics to which the organisms has been evaluated for susceptibility (Raminder *et al*., 2016).

Results and Discussion

Of the 100 swab samples collected, 30 bacteria were isolated. *Staphylococcus aureus* had frequency of 16 (53.3%), *Escherichia* coli 11(36.7%) and *Salmonella spp*. 3(10.0%) respectively (table 1). The susceptibility testing indicates that *Staphylococcus aureus* was sensitive to 3 antibiotics and resistant to 7, *Escherichia coli* was sensitive to 4 antibiotics and resistant to 6 while *Samonella spp* was sensitive to 5 antibiotics and resistant to 5 (table 2).

The results also revealed that *Staphylococcus aureus* has the MARI of 0.7, *Escherichia coli* 0.6 and *Salmonella spp*. 0.5 respectively (table 3, 4, 5).

Table 1: Distribution of bacteria in coolers

Bacterial isolates	No. of bacterial isolates
Staphylococcus aureus	16
Escherichia coli	11
Salmonella spp	3
Total	30

Table 2: The mean of inhibition (mm) of bacteria isolates to antibiotics

Organisms	CPX (10µg)	E (10µg)	LEV (10µg)	CN (10µg)	APX (30µg)	AU (30µg)	AMX (30µg)	S (30µg)	OFX (10µg)	CH (30µg)
Staphylococcus aureus	15.0	12.3	15.8	4.5	1.8	4.0	4.3	3.9	13.0	14.5
Escherichia coli	16.2	14.0	18.0	1.2	0.5	1.3	1.3	2.8	14.1	1.3
Salmonella sp	15.0	16.2	15.5	0.0	0.0	1.7	1.0	14.0	16.6	2.3

Resistant < 14, sensitive =≥ 14

Table 3: Antibiotic resistance index of *Staphylococcus aureus*

Antbiotics	Antibiotic sensitive(S)	Antibiotic(R) resistant	Antibiotic resistance index (ARI)
Ciprofloxacin	S	-	
Erythromycin	-	R	
Levofloxacin	S	-	0.7
Gentamicin	-	R	
Ampiclox	-	R	
Augmentin	-	R	
Amoxicillin	-	R	
Streptomycin	-	R	
Ofloxacin	-	R	
Chloramphenicol	S	-	
Total (10)	3	7	

Table 4: Antibiotic resistance index of *Escherichia coli*

Antbiotics	Antibiotic sensitive(S)	Antibiotic(R) resistant	Antibiotic resistance index (ARI)
Ciprofloxacin	S	-	
Erythromycin	S	-	
Levofloxacin	S	-	0.6
Gentamicin	-	R	
Ampiclox	-	R	
Augmentin	-	R	
Amoxicillin	-	R	
Streptomycin	-	R	
Ofloxacin	S	-	
Chloramphenicol	-	R	
Total (10)	4	6	

Table 5: Antibiotic resistance index of *Samonella spp.*

Antbiotics	Antibiotic sensitive(S)	Antibiotic(R) resistant	Antibiotic resistance index (ARI)
Ciprofloxacin	S	-	
Erythromycin	S	-	
Levofloxacin	S	-	0.1
Gentamicin	-	R	
Ampiclox	-	R	
Augmentin	-	R	
Amoxicillin	-	R	
Streptomycin	S	-	
Ofloxacin	S	-	
Chloramphenicol	-	R	
Total (10)	5	5	

DISCUSSION

The findings of this study have shown that *Staphylococcus aureus, Escherichia coli* and *Salmonella spp.* are present in coolers studied. These organisms are part of pathogens that causes a variety of infectious diseases. This agrees with the findings of Stratford *et al* (2016) who stated that microorganisms are often present in packaging materials as well as coolers.

The presence of these bacteria may be attributed to improper hygienic practices such as the attitude of not cleaning and disinfecting coolers by the handlers as earlier stated by Dinsesh *et al* (2018). Although, this disagrees with the identification of *Escherichia coli* as the most prevalent bacterial isolates in their study. All the bacterial isolates were resistant to more than one antimicrobial agents used with varying multiple antibiotic resistance indices. However, the organisms were all sensitive to Ciprofloxacin and Levofloxacin The resistance of these organisms maybe attributed to genetic mutation or other natural course. Goossens *et al* (2015) has reported in their work that resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another. This resistant may also occur due to the frequent use and misuse of antibiotics especially without proper prescription by physician in the study area, leading to the development of resistant bacterial strains. This is consistent with the findings of Michael *et al* (2014) who stated that in developing countries including Nigeria, there is no policy on antibiotic regulation hence, no proper prescription.

CONCLUSION

This study further confirms the growing menace of antibiotic resistance worldwide. There is need for public monitoring of resistance patterns of pathogens from storage facilities, vendors and distributors of drinks by way of enlightenment campaign on proper hygiene and regulation policy on the use of antibiotics.

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