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Assessment of Chlorine Resistant Organisms in Drinking Water (Tap Water)

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Abstract -Five strains of bacteria isolate were obtained from the drinking water samples collected from the water production section of Public waterworks in Osun State. The bacteria isolates are *klebsiella pneumoniae, Lysinibacillus fusiformis, Bacillus cereus, Pseudomonas aeruignoisa and Staphylococcus aureus.* Residua chlorine concentration from the drinking water samples were also determined using AOAC methods of analysis. Further analysis was carried out to determine the chlorine concentration at which these bacteria isolates are resistant. Some of these isolates have little resistance to chlorine at higher concentrations but *Staphylococcus aureus*, a gram positive bacteria showed higher resistivity. The resistance of the bacteria isolates to different concentrations of chlorine at different contact times shows that the grams positive bacteria have higher resistance than the grams negative bacteria. *Staphylococcus aureus* showed resistance to up onto 100 ppm concentration at 30 minutes contact time while *Klebsiella pneumonia* showed resistance to up onto 50 ppm concentration at 30 minutes contact time. Residual chlorine concentration in the treated water samples is not enough to eliminate most organisms and there is the need to increase the concentration used.

Keywords: Water, Chlorine concentration, Bacteria isolate, Resistance.

1.0 Introduction

The increasing demand for water for industrial, agricultural environmental, municipal and domestic purposes necessitate for an improvement in water treatment processes. Water quality is a topical issue in public health due to concerns emanating from the indiscriminate discharge of inadequately treated sewage into water bodies which is deleterious to human health and the environment (Khan *et al*., 2015). The lack of access to good quality water devoid borne pathogens continues to be major contributor to the disease burden, morbidity, retardation of economic growth and the wellbeing of the populace in many developing countries. In 2012, the World Health Organisation (WHO) stated and estimated that 3.4 million people, mostly children die every year form water related diseases (Martins, 2011).

Contamination of drinking water supplies can occur at the source of the water as well as in the distribution systems after water treatment has already occurred (Gerba, 2006). Sources of water contamination include naturally occurring chemicals and minerals, local land use practices (fertilizers, pesticides, concentrated feeding operations), manufacturing processes and sewer overflows or waste water releases (Hodges, 2015). The presence of contaminants in water can lead to adverse health effects like gastrointestinal illness, reproductive problems, and neurological disorders. Infant, young children, pregnant women, the elderly and people whose immune systems are compromised because of AIDS, chemotherapy or transplant medications, may be susceptible to illness from some water contaminants (Brown, 2010).

Water pathogens may be divided into three categories: bacteria, viruses and parasitic protozoa. Bacteria and viruses contaminate both surface and ground water, whereas parasitic protozoa appear predominantly in surface water (Hung, 2013). The use of an efficient water treatment system that relies on technologically compatible cost effective disinfectants that minimize the production of disinfectant by-products offers a safe margin (Baker, 2013). The purpose of disinfection is to inactivate microorganisms. One of the disinfectant chemicals used in the production of clean water is chlorine (Choi, 2010).

Chlorine is effective in killing most pathogenic bacteria, fungi and viruses. It is a strong oxidant that rapidly kills many harmful microorganisms (Janovy, 2011; Owoseni, 2013). In aqueous environments, uncombined chlorine, in form of hypochlorous acid (HClO), is an extremely potent bactericidal and virucidal agent at concentrations less than 0.1 mg/litre. Almost all water systems that disinfect water use some type of chlorine – based process, either alone or in combination with other disinfectants (Monba, 2017). This study was carried out to assess chorine resistant organisms in treated water (tap water) and to determine the chlorine lethal dosage for the treatment of some chlorine resistance organisms.

2.0 Materials and Methods

The water samples were collected from public water treatment plant in Osun state Nigeria. Materials used are sterilized while analytical grade chemicals and Reagents are used. Digital weighing instruments. measuring cylinder, petri dishes, wire loop, test tubes, *McCartney* bottles .

2.1 Microbiological Sampling and Taxonomic Identification

A portion of water sample added to MacConkey's broth, both double and single strength, which contains peptone (20 g), bile salt (5 g), neutral red (0.06 g) in one litre distilled water. For double strength, the ingredients were doubled and water volume was kept constant from positive tubes. A loop-full of suspension was streaked on nutrient agar and incubated at 35 ^{0}C for (24 hours) and colonies were enumerated and transferred to nutrient agar for cold storage (4 ^{0}C) and subsequent taxonomic identification was carried out. Colony morphology and characterisation was carried out on each isolate. The cultural and morphologic characterisation are followed by *gram* staining, motility, biochemical test (which include catalase, oxidase, indole and methyl red and voges proskaver, citrate utilisation, fermentation of carbohydrate (glucose, lactose, mannitol,, sucrose and xylose) and by using analytic profile index system.

2.1.2 Determination of Bacteria Resistance to Chorine Concentrations

The bacterial isolates were incubated into disk assay broth containing (per litre of distilled water), 0.5 g of tryptone, 0.5 g of yeast extract and 0.5 g of dextrose, were incubated at 35^{0}C for 24 hours so that the young cultures could be in the exponential growth phase. The culture was then diluted into fresh disk assay broth to barely visible turbidity. This corresponded to an optical density of 0.5 using a 600 nm. This must have 1 x 10^{-6} to 1 x 10^{-8} per ml. The stock solution of chlorine was prepared daily from Sodium hypochlorite solution, 1% in distilled sterile water. Dilutions of 1 to 5 mg/L were prepared from this solution with nutrition broth as diluting medium. Cultures of isolated strains were inoculated in test tubes with different concentrations of sodium hypochlorite solution. The cells remained in contact with chlorinated medium for 10, 20, and 30 minutes, the cultures were then inoculated in plates with nutrition agar and incubated at 37 ^{0}C for 24 hours. The presence of growth was considered a positive result.

3. Results and Discussion

The bacterial strains isolated from the treated chlorinated water were biochemically characterised and identified according to their cell shape, gram reaction, catalase, ureases, sulphide, citrate, methyl red, indole and motility levels, mannitol, galactose, sucrose and fructose as shown in table 1.0. Five strains of bacteria isolates from the treated chlorinated water were: *Staphylococcus aureus*, *Pseudomonas aeruignosa*, *Bacillus cereus*, *Lysinibacillus fusiformis* and *Klebsella spp*.

In-vitro resistance to chlorine of the bacteria isolates at different concentrations of NaOCl. Different concentrations of NaOCl was prepared to determine the concentration at which the bacteria isolates, will be sensitive to have no growth to determine the ones which are more) resistance to chlorine. The concentrations are: 1 mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5 mg/L. The resistivity and sensitivity of these bacteria isolates to the above mentioned concentrations were show in table 2. A total of five bacteria isolates were identified as presented in table 1.2 some of the isolates are of little resistance to chlorine but *Staphylococcus aureus* and *Klebseilla pneumoniae* are of higher resistance to chlorine. This may be as a result of point of collection of the sample or improper hygienic treatment of the water during production and improper storage (Moses, 2015).

4. Conclusions

The results obtained from this study showed that bacteria stains isolated from water production in the water treated plant have high microbial load which have the capacity of exposing the communities supplied with this water to serious health issues. Most of the organisms found in the treated are gram positive organisms most of which non pathogenic. These organisms were however eliminated at higher concentrations of chlorine solutions.

Table 1: Biochemical Characterization of the Bacteria Isolates and their Identification

ISOLATE CODE	CELL SHAPE	GRAM REACTION	CATALASE	UREASE	SULPHIDE	CITRATE	METHYL RED	INDOLE	MOTIITY	STARCH	GLUCOSE	SORBITOL	LACTOSE	MANNITOL	GALACTOSE	SUCROSE	FRUCTOSE	ORGANISMS
WA 1	ROD	+	+	-	-	+	-	-	+	-	NC	NC	NC	NC	A	NC	NC	*Lysinibacillus fusiformis*
WA 2	ROD	+	+	-	-	+	-	-	-	-	A	NC	NC	NC	A	NC	A	*Bacillus cereus*
WA 3	ROUND	+	+	+	-	+	+	-	-	-	A	A	A	A	A	A	A	*Staphylococcus aureus*
WA 4	ROD	-	+	-	-	+	-	-	+	-	A	NC	NC	A	NC	NC	NC	*Pseudomonas aeruginosa*
WA 5	ROD	-	+	+	-	+	-	-	-	-	A	A	A	A	A	A	A	*Klebsiella Pneumoniae*

Key: + = Growth, - = No growth, NC = No change, A = Acid Produ

Table 2: In Vitro Resistance Bacteria Isolates to Different Chlorine Concentrations

	CHLORINE SOLUTION CONCENTRATIONS (mg/L)																	
Bacteria isolates	1 mg/L			2 mg/L			3 mg/L			4 mg/L			5 mg/L			6 mg/L		
	10MINS	20MINS	30MINS	10MINS	20MINS	30MINS	10MINS	20MINS	30MINS	10MINS	20MINS	30MINS	10MINS	20MINS	30MINS	10MINS	20MINS	30MINS
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Bacillus cereus	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumonia	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Pseudomonas aeugirosa	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Lysinibacillus fusiformis	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

KEY: (+) Mean growth of bacteria, (-) mean no growth of bacteria

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