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Full Length Research paper

Microbiological analysis of some vended sachet water in Ogbomoso, Nigeria

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Water borne bacterial pathogens were isolated from sachet water vended in Ogbomoso, Oyo State, Nigeria. The isolates were characterized and identified *as Bacillus subtilis, Bacillus alvei, Pseudomonas putida, Pseudomonas fluorescens, Bacillus cereus, Enterobacter aerogenes* and *Proteus mirabilis.* The antibiotic susceptibility profile of the seven isolates was determined and it was discovered that 59.3% was found sensitive to the commercial antibiotic disc used while 40.7% was resistant. The effect of temperature, pH and sodium chloride concentration on the growth rate of isolates was investigated. It was found that as temperature of incubation increased from 50 to 80°C, the rate of growth of all the isolate decreased and as the pH of the growth medium increased from 3 to 9, the rate of growth of all the isolates also increased. As the concentration of sodium chloride increased from 2 to 5%, the rate of growth of isolates also reduced.

Key words: *Bacillus subtilis*, *Bacillus alvei*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Enterobacter aerogenes* and *Proteus mirabilis*, sachet water, pH, temperature and sodium chloride.

INTRODUCTION

Water related diseases continue to be one of the major health problems globally. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices (Tortora et al., 2002). Therefore, maintaining a safe drinking water remains essential to human health as transient bacteria contamination may have implication well beyond a period of acute-self limited illness. All living organisms require a wide variety of inorganic compounds for growth, repair, maintenance and reproduction. Water is one of the most important, as well as one of the most abundant of those compounds and it is particularly, vital to living organisms (Tortora et al., 2002). Within the cell, water is the medium for most chemical reactions. It makes up at least 5 - 95% of every cell and the average being between 65 - 75%. In addition, water has been traced to be one of the ways by which humans could be infected with various kinds of diseases some water borne diseases include typhoid fever, cholera, bacillary dysentery and so on. In water borne infections, pathogens are usually spread by water contamination with untreated or poorly treated sewage (Tortora et al., 2002).

Water is one of the most essential needs for the continued existence of all living organisms on earth. The day-to-day activities of all living organisms required water in whatever form. It is effectively and efficiently put into use by plants, animals, microorganisms and man. In the microbial world, no single microorganism has been discovered to be active at the extreme lack of water for the singular reason that man cannot exist without water, it is of paramount importance to monitor domestic water supply (Sofola and Lawal, 1983).

In many developing countries, availability of water has become a critical and urgent problem and it is a matter of great concern to families and communities depending on non-public water supply system (Okonko et al., 2008). Increase in human population exerts an enormous pres-

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sure on the provision of safe drinking water especially in developing countries (Okonko et al., 2008). Unsafe water is a global public health threat, placing persons at risk for a host of diarrhea and other disease as well as chemical intoxication (Hughes and Koplan, 2005). Unsanitary water has particularly developing effects on young children in the developing world. Each year greater than 2 million persons, mostly children less than 5 years of age, die of diarrhea disease (Hughes and Koplan, 2005). For children in this age group, diarrhea disease accounted for 17% of all death from 2000 to 2003 (WHO, 2005), ranking third among causes of death, after neonatal causes and acute respiration infections (WHO, 2005).

Water in nature is seldom totally pure. Rainfall is contaminated as it falls to earth, the combustion of fossil fuel put sulphur compound responsible for acid precipitation in the air. Water that moves below the ground's surface undergoes filtration that removes most organisms. For this reason, water from springs and deep wells are generally of good quality. The most dangerous form of water pollution occurs when feacal contaminant like *Escherichia coli* enter the water supply. Contaminants ingested into water supply cause many diseases. Examples of such pathogens are *Salmonella* spp, *Shigella* spp, *Vibrio cholerae* and *E. coli* (Tortora et al., 2002).

Industrial and agricultural chemicals leached from the land, enter water in a great amount and they could be resistant to biodegradation. Apart from this, rural water often have excessive amount of nitrite from microbial action on agricultural fertilizers (Lellan et al., 2001). When ingested nitrite competes for oxygen in the blood (Lellan et al., 2001).

To attain a safe water supply to various communities, an understanding of water that is microbiologically and chemically certified is therefore imperative. Above all, to ensure that the microbiological characteristic of drinking water is safe for human consumption, the Nigeria based National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO), recommended that potable water for human consumption should not contain any microorganism that is known to be pathogenic and the coliform number per 100 ml of water must be zero (WHO, 1984). However, it may contain three coliform per 100 ml of water sample in occasional samples (WHO, 1984).

Many people in rural and urban communities rely on sachet water and/ or borehole water as the source(s) of their drinking water supply. The integrity of these sachet waters before is doubtful, in fact, unconfirmed report abounds that most of the vendors do not treat their sachet waters before selling to the public. This become a concern for public health workers and any right-thinking individual when one consider the fact that public including nursing mothers patronize these vendors to procure water for their small children (Shear et al., 1995).

Various water borne bacteria can cause significant illness. Illness most often results from ingestion of contaminated water or seafood, with gastrointestinal entry of pathogens or their products. The skin and soft tissue are also common entry point for water borne bacteria (Czachor, 1992). Microorganisms that cause cholera, severe diarrhea, and other illness are often present in huge numbers in infected human faceas. If drinking water is contaminated with these dangerous microbes, the illnesses can results and these illnesses can spread easily to others. Diarrhea infection spread not only through water supplies but also through contaminated food, utensils and fingers. It is very difficult to control diarrhea, without a reliable supply of safe water to maintain hygiene, this reliable supply of safe water is, most often beyond, the reach of people and they rely on these cheap vended sachet water (Tibetts, 2000).

Earlier investigation conducted on the safety of drinking water has show that water on the market is of good microbiological quality while the quality of some factory bagged sachet and hand-filled polythene bagged drinking water was noted to be doubtful (Obiri-Danso et al., 2003). This observation was based on studies carried out on water sample to ascertain the presence of heterotrophic bacteria, indicators of fecal contamination (total coliform, fecal coliforms and *Enterococci*) and for lead, manganese and iron. Lack of information on pathogenic and parasitic microorganisms in sachet water on our market creates some uncertainties in our understanding of the overall quality of drinking water on our markets. To bridge this information gap, there is an urgent need for the determination of protozoan and helminthes organisms associated with drinking water in our communities (Steiner et al., 1997).

The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority, this is so because studies have attributed several disease outbreaks to untreated or poorly treated water containing bacteria pathogen that have been isolated from sachet water (Evison and James, 1973).

Hence, the objectives of the study are to; isolate and characterize microorganisms from sachet water in Ogbomoso, Oyo State, Nigeria, determine the antibiotic susceptibility profiles of the isolates and to carry out physiological studies on the isolates.

MATERIALS AND METHODS

Collection of samples

Different sachet water samples were collected in Ogbomoso, Oyo State. All samples were bought from the vendors in Ogbomoso and taken to the laboratory for analysis.

Culture media

The culture media used include nutrient agar, Eosin Methylene blue agar, MacConkey agar, Salmonella /shigella agar. The medium was prepared according to the manufacturers specification. These media were sterilized in an autoclave at 121ºC for 15 minutes.

Isolation of microorganisms

A 1 ml of each sachet water sample was serially diluted and 1 ml of an appropriate dilution was inoculated on sterile MacConkey, nutrient agar, salmonella /shigella agar and the plate was incubated for 24 h at 37°C. After 24 h, sterile wire loop was used to pick the isolates from the plate and was streaked on a freshly prepared nutrient agar and then incubated for 24 h at 37°C in order to get pure culture. After the incubation for 24 h, distinct colony was picked aseptically and cultured on a fresh nutrient agar slant and incubated for 24 h at 37ºC. After 24 h the slant was then stored in a refrigerator at -4ºC. The routine laboratory method of Cruickshank et al. (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Antibiotic susceptibility test

Sterile nutrient agar medium was poured into sterile petri-dishes and allowed to solidify. A suspension of the isolated organisms was transferred into petri-dishes accordingly and swab over the entire plate, it was then incubated for 1 h at 37ºC and a forcep was used to transfer each antibiotic disc to the plate and incubated for 24 h at 37ºC.

Survival of isolates at different temperatures ranges

Nutrient broth was prepared and dispensed into series of screw-capped bottles and sterilized. It was allowed to cool and the test organisms were inoculated into it, then incubated at different temperature ranges (50, 60, 70 and 80°C) for 24 h after which cecil 2031 (automatic) spectrophotometer was used to detect increase or decrease in turbidity of the growth medium.

Growth of isolate at different pH ranges

Nutrient broth was prepared and the pH was adjusted using 0.1 M phosphate buffer of different pH to adjust the pH of the broth to 3.0, 5.0, 7.0, and 9.0. It was then dispensed into screw capped bottles and then sterilized in the autoclave at 121ºC for 15 min. After cooling, the various test isolates were inoculated into it and incubated at 30ºC for 48 h. Growth was detected by increase turbidity using Cecil 2031 (automatic) spectrophotometer. Uninoculated tubes serve as control. This test was done to detect the best pH that favors growth and metabolism as indicated by the increased turbidity (Schillinger and Lucke, 1989).

Growth of isolates in different concentration of NaCl

Nutrient broth containing 2% (w/v), 3 %(w/v), 4% (w/v) and 5% (w/v) NaCl was prepared and sterilized at 121ºC for 15 min. A 20 ml of the broth was the dispensed into sterile screw capped vials aseptically. After cooling, the tubes were inoculated with the test organisms and incubated for 24 h at 30 ºC. Increased turbidity of the medium was recorded as positive for growth while a negative was associated with no turbidity. Uninoculated tubes serve as control (Shillinger and Lucke, 1987).

RESULTS

A total of fourteen organisms were isolated from the sachet water vended in Ogbomoso (Table 1). The isolates were initially differentiated on the basis of the cultural and morphological studies after which they were subjected to various biochemical tests and were identified with the aid of Bergey's Manual of Systematic Bacteriology. The isolates were identified to be *Bacillus subtilis*, *Bacillus alvei*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus cereus*, *Enterobacter aerogenes* and *Proteus mirabilis*.

Table 1. List of sources of isolates.

Samples code	Isolates
DER	*Bacillus subtilis*
ENU	*Bacillus subtilis*
GLO	*Pseudomonas putida*
GLO	*Enterobacter aerogenes*
CHAM	*Pseudomonas fluorescens*
CHAM	*Pseudomonas putida*
MARYK	*Bacillus alvei*
HEK	*Bacillus cereus*
HEK	*Proteus mirabilis*
FED	*Enterobacter aerogenes*
FED	*Bacillus alvei*
T.K	*Pseudomonas fluorescens*
IFESO	*Bacillus alvei*

Antibiotics sensitivity/resistance test of the entire isolated organism was determined by agar diffusion method, it was then observed that most of the isolates were resistant to streptomycin, chloramphenicol and erythromycin except *B. alvei* and *P. mirabilis*. All the isolates were sensitive to ciprofloxacin, ofloxacin and gentamycin. Most of the isolates were sensitive to pefloxacin except *P. fluorescens*. Also most of the isolates were sensitive to cotrimoxazole except *B. subtilis* and *B. alvei*. Almost all the isolates were resistant to nitrofuratoin except *B. cereus*. *P. putida* and *B. cereus* were sensitive to Augmentin with zones of inhibition of 17.5 and 13.0 mm, respectively. Most of the isolates were sensitive to Amoxylin except *B. alvei* and *P. mirabilis*. Also most of the isolates were sensitive to tetracycline except *B. subtilis, B. alvei* and *P. mirabilis*. *B. subtilis*, *B. alvei*, *B. cereus* and *P. mirabilis* were sensitive to ceftriazole with zones of inhibition of 13.5, 14.0, 13.5 and 13.5 mm, respectively but the rest were found to be resistant to ceftriazole (Table 2).

Rate of growth of the isolates at different temperature ranges was also investigated and it was discovered that as the temperature increases the rate of growth of the isolates decreases. The temperatures studied included 50, 60, 70 and 80°C. Spectrophotometric reading of wavelength of 560 nm showed that *B. subtilis* decreased in number of cells to 0.194 from 0.988 nm, *B. alvei* from 1.058 to 0.113 nm, *P. putida* also decreases from 1.154 to 0.077 nm and *P. mirabilis* also decreased from the 1.083 to 0.113 nm (Figure 1).

Table 2. Antibiotics susceptibility profile of the isolates.

Isolates	STR	CHL	ERY	CPX	OFL	GEN	PEF	COT	NIT	AUG	AMX	TET	CEF
Bacillus subtilis	-	-	-	22.0	16.5	14.0	21.0	-	-	-	11.5	-	13.5
Bacillus alvei	17.5	21.5	13.0	14.0	16.0	13.5	17.5	-	-	-	-	-	14.0
Pseudomonas fluorescens	-	-	-	18.5	18.5	17.5	-	19.5	-	-	19.5	15.5	-
Bacillus cereus	-	-	-	20.0	19.0	16.0	21.5	17.0	19.0	13.0	19.5	15.5	13.5
Proteus mirabilis	19.0	19.0	18.0	20.5	19.5	17.5	17.0	15.5	-	-	-	-	13.5
Enteroba-ter aerogenes	-	-	-	15.0	17.0	13.5	20.0	18.0	-	-	17.0	13.0	-
Pseudom-nas putida	-	-	-	15.0	17.5	15.0	16.5	11.5	-	17.5	15.0	14.5	-

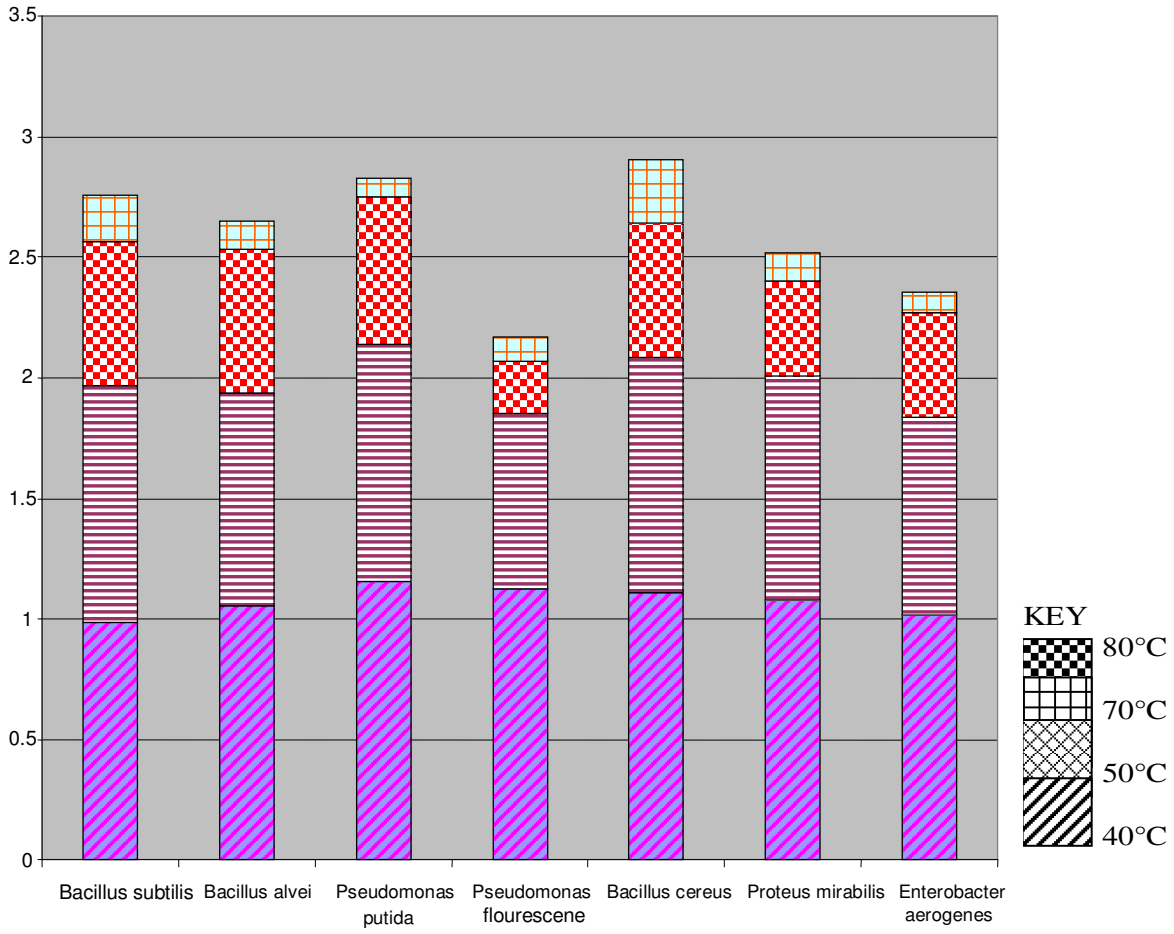

Figure 1. Rate of growth of isolates at different temperature (OD at 560 nm).

The survival of isolates in different pH was monitored using spectrophotometer at a wavelength of 560 nm and the optical density (OD) readings showed that as the pH was tending from acidic medium to alkaline medium, growth rate of all the isolates increased. It was discovered that as the pH increased from 3 to 9, *B. subtilis* increased from 0.269 to 0.958 nm, *B. alvei* increased from 0.068 to 0.980 nm, *P. putida* increased from 0.369 to 1.623 nm, *P. fluorescens* increased from 0.612 to 0.894 nm (Figure 2).

The rate of growth of isolates was monitored in different concentrations of NaCl also by using spectrophotometer at a wavelength of 560 nm and the optical density (OD) readings showed that as the concentration of sodium chloride was increased, the growth rate of all the organisms decreased. As the concentration increased from 2 to 5%, *B. subtilis* reduces from 1.058 to 0.159 nm, *B. alvei* reduces from 1.312 to 0.342 nm, *P. putida* reduces from 1.268 to 0.145nm, *B. cereus* reduced from 1.069 to 0.090 nm; this result shows that the higher the

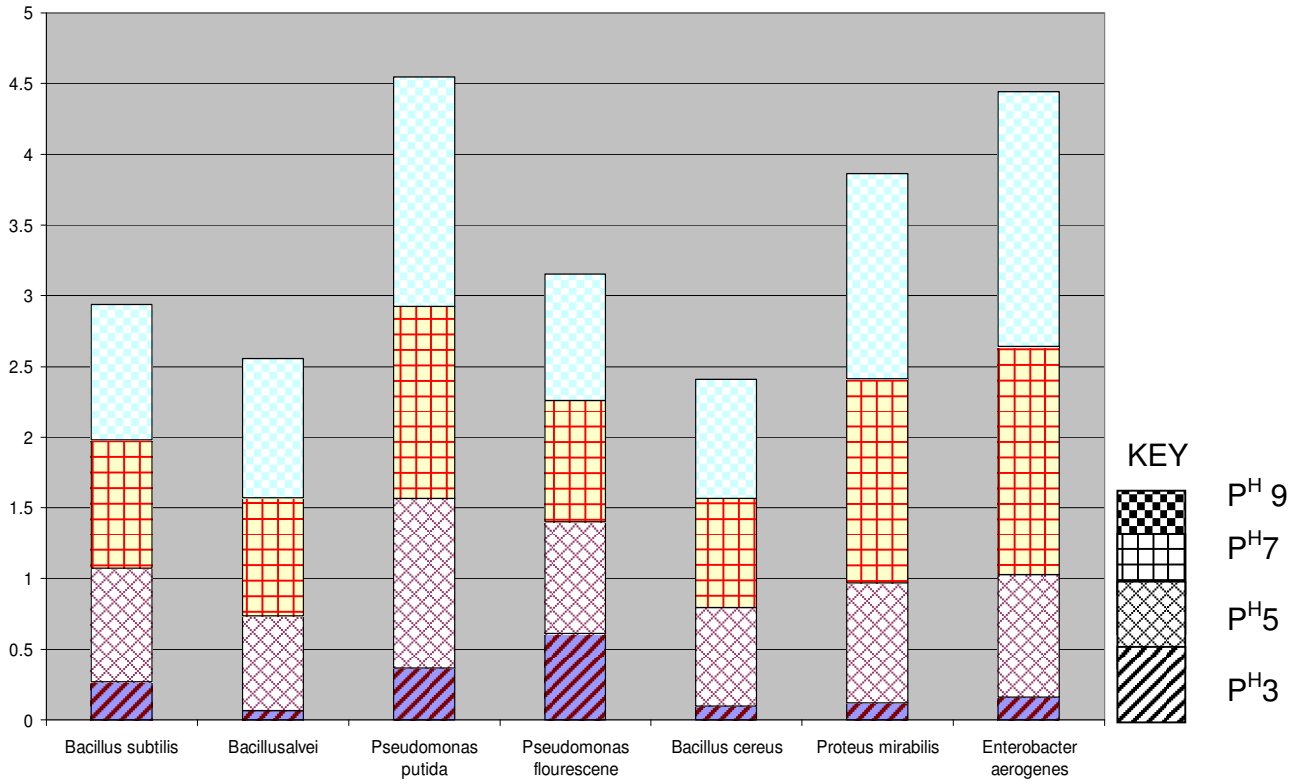

Figure 2. Survival of isolates at different pH (OD at 560 nm).

concentration of sodium chloride the lower the growth rate of the isolates (Figure 3).

DISCUSSION

All the sachet water samples collected were contaminated with bacteria; which included *Bacillus* spp., *Pseudomonas* spp., *E. aerogenes* and *P. mirabilis* and were present in different proportion. These bacteria have been implicated in water related diseases (APH, 1998).

The presence of *Bacillus* species in the sachet water could be as a result of contamination from poor staff handling during processing of the water samples (Okon et al., 2003). *Bacillus* spp., produces enterotoxin which could be deadly when ingested into the body. Also the presence of *Pseudomonas* spp., in this study, a pathogenic organism renowned for its high resistance to antibiotics, is a cause for concern (Okon et al., 2003). *E. aerogenes* and *P. mirabilis* has also been reported by Edema et al. (2001) in a study on the bacteriological quality and safety of sachet water, and attributed to burst pipes along distribution lines of drinking water or unhygienic handling of water right from treatment plant used in the production of such water (Edema et al., 2001). Presence of these bacteria in water may be unnoticed even in transparent packaged water and the presence of these microorganisms may pose a potential risk to consumers. Even the consumption of such contaminated water may facilitate the widespread of infections and can ultimately lead to outbreak of epidemic (Zuccato et al., 2000).

The degree of resistance and sensitivity of these bacteria to antibiotics differs, 59.3% was found sensitive to the antibiotics mentioned in the result and 40.7% was resistant to the antibiotics. The relatively high level of resistance to antimicrobial agent could be a reflection of misuse or abuse of this agent in the environment (Abbar and Kaddar, 1991). Bacteria becomes resistant to antimicrobial agents by a number of mechanisms which are; production of enzymes which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of antibiotics and development of metabolic pathways by bacteria which enable the site of an antimicrobial action to be by passed (Abbar and Kaddar, 1991).

As the temperature was increased the survival of the organisms was reducing for all the isolates. *Bacillus* species optimum growth temperature ranged between 55 and 65°C, *P. mirabilis* and *E. aerogenes* grow at optimum temperature of between 30 and 40°C, while *Pseudomonas* species grow at the optimum temperature of between 30 and 49°C, this implies that these the bacteria will ultimately grow and metabolize best at room temperature (Stanley et al., 1968).

The pH values of these bacteria are within the range

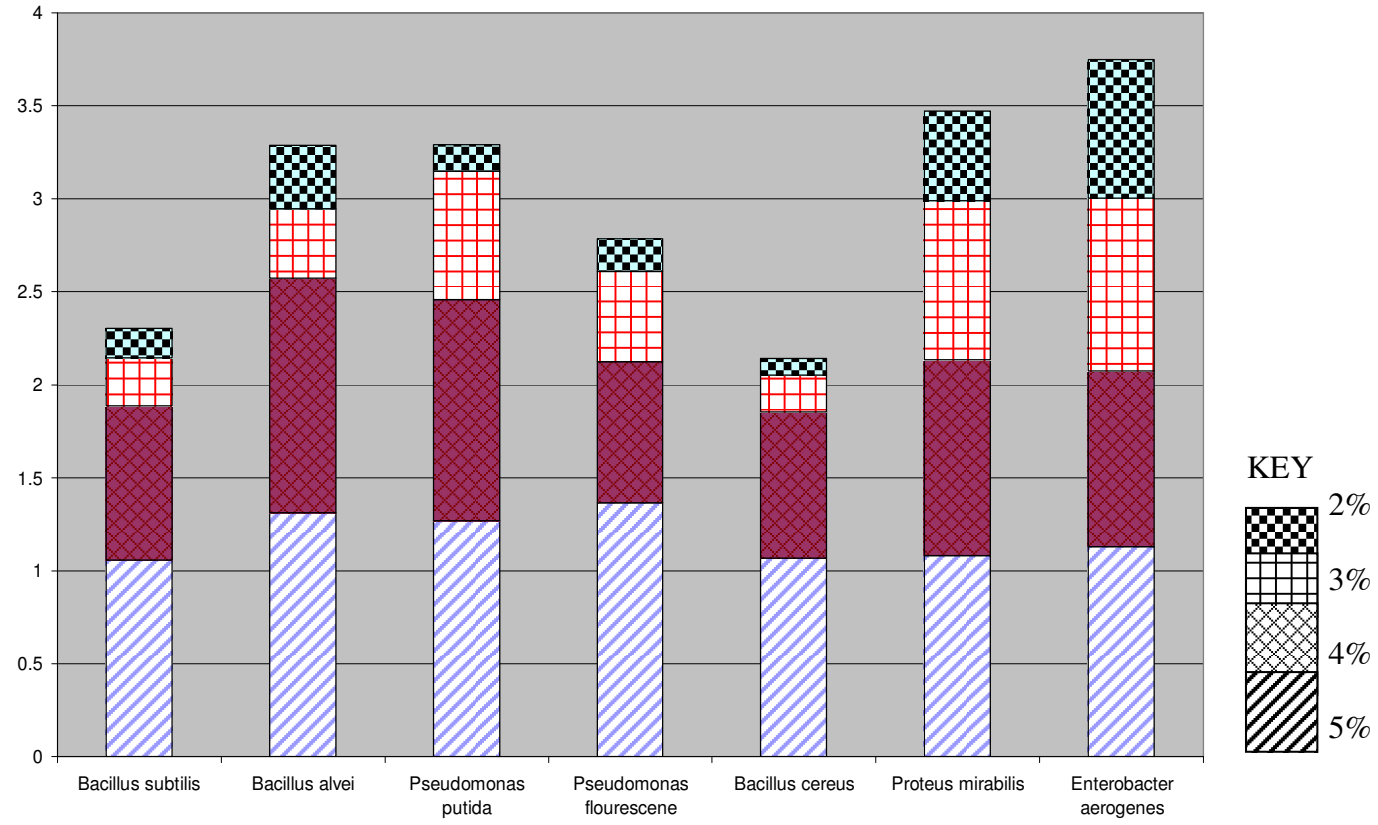

Figure 3. Growth of isolates in different concentrations Of NaCl (OD at 560 nm).

reported by Okonko et al. (2008) and Medra et al. (1982). Most microbes grow best at pH value of between 6.6 - 7.5, while few grow below pH of 4. The optimum pH required by bacteria varies and sensitive to changes, thus a fluctuation in optimum pH may lead to a change in the metabolism of the bacteria (Edema et al., 2001).

Conclusively, the presence of bacteria in this study may be as a result of improper handling, processing and purification procedures, unhygienic handling after production. Water with such bacteria are not safe for human consumption hence, the water source should be re-examined by the NAFDAC (Tortora et al., 2002).

Also microbiologically water is required for drinking, recreation and industrial uses as stipulated by the WHO and NAFDAC drinking water standards. Brenner et al. (1996) suggested that 99.8% of death in developing countries is due to unhygienic water and sanitation. Besides, the sources of untreated drinking water could be veritable reservoir of several other opportunistic pathogens of human and chemical poisoning. To reduce contamination, further investigation on sachet water is recommended. Assessment of water quality at some important stages of production; pre-production, production and postproduction stages at the factories is therefore, suggested in order to ensure their quality and safety.

Therefore, all water that fails NAFDAC and WHO regulations should be retreated before they are released to the public for human consumption. Also NAFDAC should intensify effort on batch number, production date and expiry date of all these sample vended in public.

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