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Full Length Research Paper

Antibiotics sensitivity patterns of *Escherichia coli* and *Aerobacter aerogenes* isolated from well water in Ile– Ife, Nigeria

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Ten wells were arbitrarily chosen for study in Sabo area in Ile-Ife, Nigeria. The water samples were collected to estimate the extent of faecal pollution and the probable health hazard the use of these well waters can impound on the community. Coliform count was determined using the Most Probable Number (MPN) Technique. The indicator organisms in this study were *Escherichia coli* and *Aerobacter aerogenes*. Samples of water obtained from the wells were considered unsafe for human consumption, especially when used untreated, because they contain a high density of coliforms. Various morphological, cultural characteristics and biochemical tests were also carried out on the indicator organisms. *E. coli* was sensitive to cotrimoxazole, streptomycin, tetracycline, colistin, gentamicine and nalidixic acid but resistant to nitrofurantoin, ampicillin, cephalocidine, sulphafurazole, carbenicillin and sulfamethazole while *Aerobacter aerogenes* was sensitive to colistin, gentamicine and nitrofurontoin but resistant to the remaining antibiotics of the Gram negative disc. For the U4-disc, *Escherichia coli* was susceptible to colistin sulphate but resistant to all the other antibiotics. *Aerobacter aerogenes* was resistant to all the U4 antibiotics discs. It is recommended that a major programme which will intimate rural and urban dwellers of the sources, risk and consequences of water pollution and water-borne diseases should be carried out in the near future.

Key words: Antibiotics sensitivity patterns, *Escherichia coli*, *Aerobacter aerogenes*, well water.

INTRODUCTION

Water is essential to life and it is of fundamental importance to all living organisms. Water constitutes about 90% by weight of the human body (William et al., 2002). The availability of drinking water is an indispensable feature for preventing epidemic diseases and improving the quality of life (Borchard et al., 2004). However, most rural villages in developing countries have poor access to safe clean water supply and sanitary facilities. As water becomes scarce, people tend to use any locally available source without any form of pre-treatment which however, leads to obvious problems of health and sanitation (Kritof, 1997). According to the World Health Organization (WHO, 1981), 80% of all diseases are attributed to unsafe water with about $1^1/_4$ billion people in the world suffering from major related diseases at any time (Odeyemi, 1988). However, water for human consumption and use are derived from different sources which can be natural or artificial. Natural sources include rainfall, well, bore-hole, spring, river, stream, sea, ocean and lake while artificial water sources include distilled water, purified or treated water (Nash,

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Table 1. Description of well water sources in Sabo area of Ile-Ife, Nigeria.

Well no.	Depth (Ft)	Well opening	Well cover	Concrete internal ring
1	17	Raised	Covered	Present
2	24	Unraised	Covered	Present
3	18	Raised	Covered	Absent
4	24	Raised	Covered	Present
5	24	Raised	Covered	Absent
6	20	Unraised	Covered	Present
7	22	Raised	Covered	Present
8	18	Unraised	Covered	Absent
9	24	Unraised	Covered	Absent
10	22	Unraised	Covered	Present

1993).

Pollution in water occurs from a variety of sources and contamination with pathogenic organisms remains a major cause of epidemic diseases (Bowman, 1994). The objective of the chemical and bacteriological examinations of water is to maintain good quality of drinking water and also to ascertain its safety, which according to the World Health Organization (WHO, 1984), should contain a maximum of one coliform bacterium per 100 ml of water. In view of the fact that shortage of pipe-borne water in Ile-Ife, Osun State, Nigeria is now the rule rather than the exception, majority of the inhabitants now depend on well water. The study was designed to examine some wells in Ile- Ife for the presence of coliforms which are widely used in addition to other bacteria as indicators of water pollution.

MATERIALS AND METHODS

A total of ten wells were sampled from Sabo area of Ile-Ife, Nigeria. Four of the wells were approximately 24 ft deep while the others were within the range of 17 and 22 ft. Six of the wells were with internal ring concrete while the others were without. Five of the well openings were at ground level while the remaining five were raised above ground level. A brief description of the well water sources is presented in Table 1. Seven of the ten house holds use their well water for washing, cooking and drinking while the remaining three use theirs for only washing and cooking.

Sample collection

Water samples were collected from ten different wells in the same geographical location with the use of sterile sample bottles. A strong thread was attached to the neck of each sterile bottle and gently released into the well; the opened bottle was allowed to sink below the water and was pulled up after observing there were no more bubbles from the bottle. The bottle was gently raised out of the well without allowing bottle to touch the sides of the wells. The caps were carefully replaced and the sample was transported in ice bath to the laboratory for immediate analysis.

Media preservation

The media used were MacConkey broth, nutrient agar, Eosin Methylene Blue (EMB) agar, nutrient gelatin agar and Diagnostic Sensitivity Test (DST) agar. Each medium was prepared according to the manufacturer's specification.

Outline of tests performed (MPN)

The method used for the detection and enumeration of coliform was the multiple tube fermentation technique, which involved three tests as follows: Presumptive, confirmatory and completed tests. Biochemical tests carried out include Gram stain, citrate utilization test, indole production test, Methyl red – Voges Proskaeur` test and sugar fermentation tests.

Antibiotics susceptibility test

The medium used was Diagnostic Sensitivity Test (DST) agar (Oxoid). It was prepared according to the manufacturer's specification. Antibiotic multidisks (Gram negative disc and U4 – discs) (Oxoid) were used.

RESULTS

Most probable number of coliform bacteria

A total number of ten wells were examined for the presence of coliforms. The results showed that the wells contained most probable number (MPN) of bacteria ranging from 43 to more than 1,100 bacteria per 100 ml. The samples from Well numbers 1, 4, 7 and 10 yielded relatively low coliform counts of 15 to 53 bacteria compared with Well numbers 8 and 9 which had more than 1,100 bacteria each.

Density of *E. coli* and *A. aerogenes*

On Eosin Methylene Blue (EMB) agar, *E. coli* strains

Table 2. Most Probable Number (MPN) of coliforms in well water samples from Sabo area of Ile-Ife, Nigeria.

Sample number	Quantity of water (ml)			MPN per 100 ml
	10	1	0.1	
	No. of samples of each quantity tested			
	3	3	3	
	Number of tubes giving positive reactions (acid and gas)			
1	2	3	1	36
2	3	2	2	210
3	3	3	2	1100
4	2	0	3	16
5	3	3	2	1100
6	3	3	0	240
7	2	3	3	53
8	3	3	3	1100
9	3	3	3	1100
10	3	1	0	43

Table 3. Density of *E. coli* and *A. aerogenes* in well water.

Sample number	*E. coli* (cfu)		*A. aerogenes* (cfu)	
	Duration (h)			
	24	48	24	48
1	2	3	3	5
2	5	8	4	7
3	27	39	14	23
4	0	0	1	2
5	20	29	12	18
6	4	5	0	2
7	12	14	8	10
8	17	25	19	29
9	22	30	19	32
10	0	0	4	6

appeared as greenish metallic sheen colonies while *Aerobacter aerogenes* appeared as large pinkish mucoid colonies. Table 2 shows that *E. coli* was present in eight of the wells but absent in two of them (Well numbers 4 and 10). The results also show that *Aerobacter aerogenes* was present in all the well water samples (Table 3).

Morphological and cultural characteristics of bacteria isolated from well water

On nutrient agar, organisms identified as *E. coli* appeared colourless with a smooth surface. The second organism identified as *A. aerogenes* appeared dome-shaped with glistered colonies on Eosin Methylene Blue (EMB). Both organisms were Gram negative bacilli (Table 4).

Biochemical reactions

Methyl red, Voges Proskaeur and catalase test

After 5 days of incubation at 37°C, it was observed that the strains of *E. coli* isolated were methyl red, Voges Proskaeur and catalase test while *A. aerogenes* were negative for methyl red test, positive for Voges Proskaeur test and positive for catalase test (Table 5).

Citrate utilization and indole production

Some organisms have the ability to utilize citrate as their sole carbon source. This test showed that the *E. coli* lacked the ability to utilize citrate as a sole source of carbon but indole test positive. *A. aerogenes* was able to utilize citrate as its sole carbon source but indole test negative (Table 5).

Fermentation of sugars

E. coli isolated fermented sugars thus producing acid and gas except for starch while *A. aerogenes* fermented all the sugars producing acid and gas respectively. Gas production was not found for the strain of *E. coli* that fermented sucrose (Table 5).

Antibiotics susceptibility test

This test indicated that *E. coli* was sensitive to cotrimoxazole, streptomycin, tetracycline, colistin, gentamicin and nalidixic acid but resitant to nitrofurantoin, ampicillin, cephalocidine, sulphafurazole, carbenicillin and sulfamethazole while *A. aerogenes* was sensitive to

Table 4. Morphological and cultural characteristics of bacteria isolated from well water.

Features \ Characteristics	*E. coli*	*A. aerogenes*
Growth on nutrient agar	Circular, low convex, smooth surface, colourless	Dome-shaped glistering colonies
Growth on Eosin Methylene blue	Dark centre colonies with greenish metallic sheen	Large pinkish mucoid colonies with no metallic sheen
Gram stain	Gram negative bacilli	Gram negative bacilli

Table 5. Biochemical characteristics of organisms isolated from water samples.

S/N	Biochemical characteristics	*E. coli*	*A. aerogenes*
1	Indole production	+	_
2	Methyl Red	+	_
3	Voges Proskauer	_	+
4	Citrate utilization	_	+
5	Catalase production	+	+
6	Galactose	AG	AG
7	Glucose	AG	AG
8	Lactose	AG	AG
9	Maltose	AG	AG
10	Mannitol	AG	AG
11	Sucrose	A	AG
12	Starch	NIL	AG

+ = Positive; A = Acid; NIL = no production - = Negative; G = Gas.

colistin, gentamicin and nitrofurantoin but resistant to the remaining antibiotics of the Gram negative disc. For the U4 disc, *E. coli* was susceptible to colistin sulphate and resistant to all other antibiotics. *A. aerogenes* was also resistant to the entire antibiotics disc (Table 6).

DISCUSSION

Water, one of the primary needs of man is exposed to many biological and chemical contaminations. This becomes more pronounced when people use water from natural sources which are not subjected to any prior treatment before it is used for cooking, washing and drinking. Examples of this source of supply are the wells on which these examinations were carried out. Water for human consumption should be free of coliforms; the World Health Organization sets the limit of <1 coliform per 100 ml drinking water (WHO, 1984). In each well water analysed in this study, the level of coliform density was far greater than the standard set by the World Health Organization; the lowest obtained being 16 coliform bacteria per 100 ml of well water. Practically, all water under natural conditions contain microorganisms; some contain many microorganisms while others contain a few. The results of this investigation showed that the quality of all the wells examined failed to meet the standard of safe drinking water since all the wells have significant coliform population. Normally water for human consumption should be free of coliform bacteria (United States Public Health Service, 1995). The presence of *E. coli* and *A. aerogenes* suggest feacal pollution, hence the quality of the wells fell strongly below the standrad of safe drinking water. Although most strains of *E. coli* are not pathogenic, their presence is indicative of the possible presence of pathogenic organisms. Water is considered safe when it is free of *E. coli* (Wanke, 1990).

The environment in which a well is located majorly contributes to its level of pollution. For a well to be safe from contamination; it should be properly constructed (that is, deep) and it should be at least 100 to 200 m away from dumping grounds, pit toilets and bathrooms (Nash, 1993). However, it was generally observed during the course of this study that most of the wells examined were subject to various types of pollution from the environment. In particular, well numbers 8, 9 and 10 were consistently surrounded by feacal materials of both human and animal origins throughout the sampling period. It was also noticed that the buckets used in drawing water in all the sites were not washed before use. These buckets could therefore easily pick up microorganisms in the soil and faecal materials from the surroundings, thereby increasing the level of feacal pollution of the wells. According to Sangonuga (1983), the cholera outbreaks of 1978 and 1980 in Ile-Ife and its environment were associated with unfavourable location of wells (relative to pit latrines in many cases), indiscriminate defecation around wells and sequential washing down of faecal matter into wells and the few surface streams from which people fetch water. It is assumed that if coliforms (which are normal inhabitants of the intestines of humans and animals) are present in water, pathogens from the tracts of infected individuals could also be present (Levin and Edelman, 1994). The high coliform density in all the wells, therefore, suggests that the well water samples in all the sites examined were not safe for human consumption (WHO, 1981).

Table 6. Antibiotics susceptibility pattern of *E. coli* and *A. aerogenes* isolated from water samples obtained from wells.

Serial no.	Disc code	Antibiotics	Concentration (µg)	Growth measurements (mm)	
				E. coli	*A. aerogenes*
1	Gram negative disc	Cotrimoxazole	25	21	0
2		Streptomycin	25	12	0
3		Tetracycline	25	12	0
4		Ampicillin	25	0	0
5		Colistin	25	31	19
6		Gentamicin	10	14	22
7		Nalidixic acid	30	25	0
8		Nitrofurantoin	200	0	9
9	U4 disc	Gentamicine	10	0	0
10		Cephalocidine	25	0	0
11		Colistin sulphate	10	18	0
12		Sulpha furazole	500	0	0
13		Ampicillin	25	0	0
14		Carbenicillin	100	0	0
15		Sulfamethazole/ trimethoprim	25	0	0
16		Tetracycline	50	0	0

The results of this investigation show that the isolated *E. coli* and *A. aerogenes* exhibited multiple resistance to a number of commonly used antibiotics such as ampicillin, streptomycin and tetracycline. The highest resistance was recorded for ampicillin. The results corroborate those of Murray et al. (1989). This resistance has become notable in most developing countries (Nigeria inclusive) among adults and children (Antai and Anozie, 1987; Lamikanra et al., 1989). Appreciable susceptibility of *E. coli* to cotrimoxazole, colistin, nalidixic acid and gentamicin was recorded. Similar observations have also been recorded (Lamikanra et al., 1989).

CONCLUSION AND RECOMMENDATION

During the course of this research, it was observed that the sanitary conditions and quality of all the wells in Ile-Ife leaves much to be desired. The high coliform density recorded in all the wells is an indication of poor sanitary conditions. Each of the wells contained coliforms above the acceptable standard limit for drinking waters by the World Health Organization. Most of the wells were located near dump grounds and pit toilets where feacal materials could easily contaminate them. In view of the fact that diseases and other hazards can emanate from water containing high number of microorganisms, the underlying problem needs urgent solution. The wells should be properly constructed (that is, they should be as deep as possible and their upper portion must be lined with an impervious material so that water from the surface will not seep into them). Mass literacy and health campaign through television, mass media, and radio will go a long way in reducing this problem in Ile-Ife and Nigeria in general.

Indiscriminate use of antibiotics in chemotherapy should be avoided to prevent the development of more resistant strains of *E. coli*, *A. aerogenes* and other pathogenic organisms. Since water is most needed in human society, any effort channeled towards improving its quality will not be too much. When achieved (improved water quality), it will go a long way in putting an end to water-borne diseases in our society.

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