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ANTIBIOTICS RESISTANCE PROFILE OF ESCHERICHIA COLI AND ENTEROBACTER AEROGENES ISOLATED FROM WELL WATERS IN AGO-IWOYE, SOUTHWESTERN NIGERIA

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ABSTRACT

Eighty percent of all diseases are attributed to unsafe water with about 11.4 billion people in the world suffering from major related diseases at various times. This study was carried out to investigate the antibiotics resistance profile of *Escherichia coli* and *Enterobacter aerogenes* isolated from well waters in Ago-Iwoye, Southwestern Nigeria. Water samples were collected from ten different wells. The multiple tube fermentation technique was employed to enumerate coliforms using MacConkey broth. Nutrient agar and ethylene methylene blue (EMB) agar were used for the enumeration of *Escherichia coli* and *Enterobacter aerogenes*. Biochemical characterization was carried out using standard methods. The disk diffusion method was used to determine the antibiotics susceptibility profiles of the bacterial isolates. Results showed that wells contained most probable number (MPN) of bacteria ranging from 43 to 1,100 bacteria per 100 ml. *E. coli* was present in eight of the wells while *E. aerogenes* was present in all the wells. Wells B and C had the highest incidence of *E. coli* with 5.0 x 10^2 CFU/100ml while wells D and J had no incidence of *E. coli.* Occurrence of *Enterobacter aerogenes* was highest in well B (4.5 x10^2 CFU/100ml), followed by well C (4.0 x10^2 CFU/100ml) while the lowest occurrence was obtained from well F having a count of 2.0 x 10^2 CFU/100ml. *E. coli* was resitant to nitrofurantoin, ampicillin, cephalocidine, sulphafurazole, carbenicillin and sulfamethazole while *E. aerogenes* was sensitive to 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. *E. aerogenes* was resistant to the entire U4 discs. The presence of *E. coli* and *E. aerogenes* suggested faecal pollution, hence the quality of the wells fell strongly below the standard of safe drinking water. Most strains of isolates showed relative resistance to antibiotics investigated in this study and this should be of great concerns to researchers.

Keywords: Antibiotics; resistance; health; risk; water; wells; coliforms.

Discipline and Sub-discipline: Microbiology (Medical Microbiology and Parasitology).

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1.0 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 [1]. The availability of drinking water is an indispensable feature for preventing epidemic diseases and improving the quality of life [2]. 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 pretreatment which however, leads to obvious problems of health and sanitation [3]. According to the World Health Organization [4], 80% of all diseases are attributed to unsafe water with about 11.4 billion people in the world suffering from major related diseases at various times [5]. However, water for human consumption and use are derived from different sources which can be natural or artificial. Natural sources include rainfall, well, borehole, spring, river, stream, sea, ocean and lake while artificial water sources include distilled water, purified or treated water [6,7]. Pollution in water occurs from a variety of sources and contamination with pathogenic organisms remains a major cause of epidemic diseases [8]. 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 [9,10], 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 Ago-Iwoye, Nigeria is now the rule rather than the exception, majority of the inhabitants now depend on well water. Water is the life wire of the body and, infact, it is the basis of life; it is a critical part of human diet. However, many water sources in developing countries are unhealthy because they contain harmful physical, chemical and biological agents. The World Health Organization (WHO) estimates that up to 80% of ill health in developing countries are water and sanitation related [11,12]. High incidence of childhood diarrhea, helminthiasis, trachoma and the overall high mortality rates are associated with poor environmental sanitation [13]. The contamination of water remains a problem of global concern contributing to high morbidity and mortality rates from water and food borne diseases [14]. *Escherichia coli* is a common member of the intestinal microflora of both humans and warm blooded animals. It is a commensal or opportunistic pathogen implicated in acute urinary tract infections (UTI) and gastrointestinal tract infections [14]. It is a consistent and predominant facultative inhabitant of the human gastro-intestinal tract, thus its regular presence in the intestine and faeces of warm blooded animals makes the bacterium an indicator of faecal pollution [15]. The presence of faecal coliforms in water indicates contaminant per se, and may indicate that the sample is unsuitable for consumption [16]. Maynard *et al.* [17] reported that the use of indicator bacteria such as faecal coliforms and *Escherichia coli* for the assessment of faecal pollution and possible water quality deterioration in various water sources is widely accepted concept in the world. Water contamination events often results from discharges from waste water treatment facilities, overflowing sanitary sewer systems, waste materials that find their way into domestic and industrial sewage and run off of animal faecal matters during storm events [10,17,18]. Exposure to contaminated water through ingestion such as drinking water, recreation or irrigation is a significant mode of transmission of gastro intestinal tract infection [19,20]. A collaborative, interdisciplinary effort to ensure global access to safe water, basic sanitation, and improved hygiene is the foundation for ending cycle of poverty and diseases [21]. Conformation with microbiological standard is of special interest because of the capacity of water to spread diseases within a large population. Although the standards vary from place to place, the objective anywhere is to reduce the possibility of spreading water-borne disease in addition to being pleasant to drink, which implies that it must be wholesome and palatable in all respects [20,22,23]. 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 [23]. Increase in human population has exerted an enormous pressure on the provision of safe drinking water especially in developing countries [24]. Unsafe water is a global public health threat, placing persons at risk for a host of diarrheal and other diseases as well as chemical. Unsanitary water, particularly, has devastating effects on young children in the developing world. Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrheal disease [25,26]. For children in this age group, diarrheal disease accounted for 17% of all death from 2000 to 2003 ranking third among causes of death, after neonatal causes and acute respiratory infections [12]. Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation conditions affecting a large part of the world's population [21,27]. An estimated 1.1 billion persons (one sixth of the world's population) lack access to clean water and 2.6 billion to adequate sanitation [12,21]. Resistant bacteria are becoming commonplace in healthcare institutions. Bacterial resistance often results in treatment failure, which can have serious consequences, especially in critically ill patients [28,29,30,31,32,33]. *Enterobacter aerogenes* cause a variety of infections, often transmitted in the hospital setting. More importantly, *Enterobacter aerogenes* has shown to display multidrug resistance due largely to mutations that encode porins (protein channels) and membrane efflux pumps that pump out antibiotics before they can harm the organism. These have been shown to be non-specific which accounts for their multiple drug resistance. Structurally unrelated molecules such as B-lactam antibiotics, quinolones, tetracyclines, and chloramphenicol are all kept at bay [34].

2.0 MATERIALS AND METHODS

2.1 Study Area

Ago Iwoye is a town in Ijebu North Local Government Area of Ogun State, Southwestern Nigeria. It is one of the most popular cities in Ogun State. It has seven divisions Idode, Imere, Isamuro, Ibipe, Imososi, Igan, Mamu and Oke Ebute. The great Olabisi Onabanjo University is situated in Ago-Iwoye. It has a population of about 300,000 people.

2.2 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 [7].

2.3 Microbiological Analysis

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. Nutrient agar and ethylene methylene blue (EMB) agar plates were inoculated with 1 ml of 10^{-2} dilution factor of each water sample using sterile pipette for the isolation and enumeration of *Escherichia coli* and *Enterobacter aerogenes*. Discrete colonies, after 48 hrs of incubation at 37^oC, were sub-cultured to obtain pure cultures and were identified according to standard procedures [35]. MacConkey broth was used for the enumeration of coliforms. Biochemical tests carried out were Gram's staining, citrate utilization test, indole production test, methyl red test, Voges Proskaeur test and sugar fermentation tests [35,36].

2.4 Antibiotics Susceptibility Testing

The Kirby-Bauer (disk diffusion) method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. Antibiotic multidisks used consisted of Gram negative discs and U4 discs (Oxoid). Gram negative discs included Cotrimoxazole (25 µg), Streptomycin (25 µg), Tetracycline (25 µg), Ampicillin (25 µg), Colistin (25 µg), Gentamicin (10 µg), Nalidixic acid (30 µg) and Nitrofurantoin (200 µg) while the U4 discs were Gentamicin (10 µg), Cephalocidine (25 µg), Colistin sulphate (10 µg), Sulpha furazole (500 µg), Ampicillin (25 µg), Carbenicillin (100 µg), Sulfamethazole (25 µg) and Tetracycline (50 µg). The medium used was Mueller Hinton (MH) agar. Pure cultures of organisms were enriched in nutrient broth and incubated at 37^0C to a turbidity of 0.5 Macfarland standards. The MH agar was inoculated by streaking using sterile cotton swab of each of the cultures. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotics and the plates were incubated inverted at 37^0C for 24 hours. Results were recorded by measuring the zone of inhibition and comparing with the NCCLS interpretive performance standard for antimicrobial disk susceptibility testing [37].

3.0 RESULTS

Table 1: Description of well water sources sampled in Ago - Iwoye, southwestern Nigeria.

Well No.	Depth (Ft)	Well Opening	Well Cover	Concrete Internal Ring
1	23	Raised	Covered	Present
2	26	Unraised	Covered	Present
3	21	Raised	Covered	Present
4	27	Unraised	Covered	Present
5	23	Raised	Covered	Present
6	23	Raised	Covered	Present
7	22	Raised	Covered	Present
8	21	Unraised	Covered	Present
9	27	Raised	Covered	Present
10	24	Raised	Covered	Present

A total of ten wells were sampled in Ago-Iwoye, Nigeria. The depth of the well ranged between 21 – 27 ft. All the wells were with concrete internal ring and were with metal cover. Seven of the well openings were raised above the knee level while the remaining three were unraised (two were just at ground level and one below knee level). A brief description of the well water sources is presented in table 4.1 and pictures presented in appendix 1. These well waters were considered potable by the households and inhabitants of the community as they were being used for drinking and other domestic purposes such as cooking, washing etc.

Table 2: Most probable number (MPN) of coliforms in well water samples in Ago-Iwoye, Southwestern Nigeria.

Sample Code	Water quantity (ml)			MPN/ 100 ML
	10	1.0	0.1	
	Number of samples of each quantity used			
	3	3	3	
	Number of tubes showing positive reactions (acid and gas)			
A	2	3	1	36
B	3	3	2	1100
C	3	3	2	1100
D	3	2	2	210
E	3	3	0	240
F	3	1	0	43
G	3	3	3	1100
H	3	3	3	1100
I	2	3	3	53
J	2	0	3	16

Table 2 showed that the wells contained most probable number (MPN) of bacteria ranging from 43 to more than 1,100 bacteria per 100 ml. Wells labelled A, F, I and J yielded relatively low coliform counts compared with wells labelled B, C, G and H which had about 1,100 bacteria each.

Table 3: Morphological and cultural characteristics of bacteria isolated from well waters in Ago Iwoye, Southweswern Nigeria

Features	*E. coli*	*E. aerogenes*
Growth on nutrient Agar	Circular, low convex, Smooth surface, colourless	Dome-shaped glistering colonies
Growth on eosin methylene blue (EMB) agar	Dark center colonies with greenish metallic sheen	Large pinkish mucoid colonies with no metallic sheen
Gram's Stain Reaction	Gram negative bacilli	Gram negative bacilli

Organisms identified as *E. coli* appeared circular, low convex and colourless with a smooth surface on nutrient agar, and as dark-centered colonies with greenish metallic sheen on EMB agar. The second organism identified as *E. aerogenes* appeared dome-shaped with glistering colonies on nutrient agar and as large pinkish mucoid colonies with no metallic sheen on EMB. Both organisms were Gram negative bacilli (Table 3)

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Table 4: Biochemical characteristics of organisms isolated from water samples in Ago-Iwoye, Southwestern Nigeria

Biochemical characteristics	*E. coli*	*E. aerogenes*
Indole production	+	-
Methyl Red	+	-
Voges Proskauer	-	+
Citrate utilization	-	+
Catalase production	+	+
Galactose	AG	AG
Glucose	AG	AG
Lactose	AG	AG
Maltose	AG	AG
Mannitol	AG	AG
Sucrose	A	AG
Starch	Nil	AG

After 5 days of incubation at 37°C, it was observed that the strains of *E. coli* isolated were positive for indole, methyl red and catalase test while *E. aerogenes* were negative for methyl red test, positive for Voges Proskaeur test and positive for catalase test. Some organisms have the ability to utilize citrate as their sole carbon source. Strains of *E. coli* isolated lacked the ability to utilize citrate as sole source of carbon but were indole-positive. *E. aerogenes* was able to utilize citrate as sole carbon source but indole- negative. *E. coli* isolated fermented sugars thus producing acid and gas except for starch while *E. aerogenes* fermented all the sugars producing acid and gas. Gas production was not found for the strains of *E. coli* that fermented sucrose (Table 4).

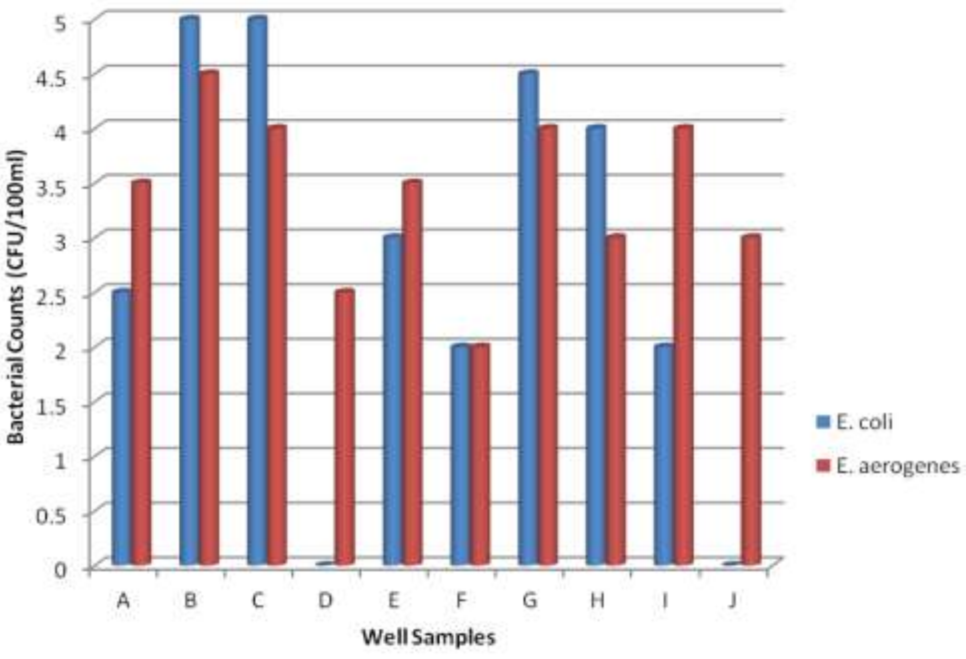

Figure 1:Occurrence of *Escherichia coli* and *Enterobacter aerogenes* in well water samples in Ago – Iwoye, Southwestern Nigeria

E. coli was present in eight of the wells and absent in two of them (wells D and L). *E. aerogenes* was present in all the wells. Wells B and C had the highest incidence of *E. coli* with 5.0 x 10^2 CFU/100ml while wells D and J had no incidence of *E. coli.* Occurrence of *Enterobacter aerogenes* was highest in well B (4.5 x10^2 CFU/100ml), followed by well C (4.0 x10^2 CFU/100ml while the lowest occurrence was obtained from well F having a count of 2.0 x 10^2 CFU/100ml ((Figure 1).

Table 5: Antibiotics resistance patterns of *E. coli* and *E. aerogenes* isolated from well water samples in Ago Iwoye, Southwestern Nigeria

Serial Number	Disc Code	Antibiotics	Concentration (μg)	Zone of Inhibition (mm)	
				E. coli	*E. 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	9
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	25	0	0
16		Tetracycline	50	0	0

E. coli was sensitive to cotrimoxazole, streptomycin, tetracycline, colistin, gentamicin and nalidixic acid but resitant to nitrofurantoin, ampicillin, cephalocidine, sulphafurazole, carbenicillin and sulfamethazole while *E. aerogenes* was sensitive to 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. *E. aerogenes* was also resistant to the entire antibiotics disc (Table 5).

4.0 DISCUSSION AND CONCLUSION

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 drinking, cooking and washing. Examples of this source of supply are the wells on which these examinations were carried out. The environment in which a well is located majorly contributes to its level of pollution. 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 sample investigated in this study, the level of coliform density was far greater than the standard set by World Health Organization; the lowest count obtained was 16 coliforms per 100 ml of well water (Table 2). Practically, all waters under natural conditions contain microorganisms; some contain many microorganisms while others contain a few. Normally water for human consumption should be free of coliform bacteria [12]. Although most strains of *E. coli* are not pathogenic, their presence is indicative of the possible presence of pathogenic organisms. Water is considered bacteriologically safe when it is free of *E. coli* [38]. However, for a well to be safe from contamination, it should be properly constructed and deep, and it should be at least 100 to 200 m away from dumping grounds, pit toilets and bathrooms [6]. However, it was observed in this study that the environments of some of the wells examined were unkept, and buckets used for drawing water in all sites were not washed on regular basis as indicated by algal growth in some of these containers. These buckets could easily pick up microorganisms in the soil and faecal materials from the surroundings, thereby increasing the level of faecal pollution of the wells. *E. coli* and *E. aerogenes* exhibited multiple resistances to a number of commonly used antibiotics such as ampicillin, streptomycin and tetracycline. The highest resistance was recorded for ampicillin (Table 5). The results corroborate those of Murray *et al.* [39] and Bello et al. [40]. In conclusion, 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. The presence of *E. coli* and *E. aerogenes* suggested faecal pollution, hence the quality of the wells fell strongly below the standard of safe drinking water as described by WHO [4]. Most strains of isolates showed relative resistance to antibiotics investigated in this study and this should be of great concerns to researchers.

5.0 RECOMMENDATION

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 faecal 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. It is clear that bacteria will continue to develop resistance to currently available antibacterial drugs by either new mutations or the exchange of genetic information, that is, putting old resistance genes into new hosts. In many healthcare facilities around the world, bacterial pathogens that express multiple resistance mechanisms are becoming the norm, complicating treatment and increasing both human morbidity and financial costs. Prudent use of antibacterial drugs, using the appropriate drug at the appropriate dosage and for the appropriate duration, is one important means of reducing the selective pressure that helps resistant organisms emerge. The other vital aspect of controlling the spread of multidrug-resistant organisms is providing sufficient personnel and resources for infection control in all healthcare centres. New antibacterial agents with different mechanisms of action are also needed. It is difficult to outsmart organisms that have had several billion years to learn how to adapt to hostile environments, such as those containing antimicrobial agents. With sufficient efforts to avoid indiscriminate use of antimicrobial agents, thereby preventing the emergence of resistant organisms, and strict attention to infection control guidelines to contain the spread of resistant organisms when they develop, we should be able to stay at least one step ahead of the next resistant plague. Since water is most needed in human society, any effort channeled towards improving its quality will not be too much. This will go a long way in putting an end to water-borne diseases in our society.

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APPENDIX 1

Well A: A raised well with metal cover and with openings at the sides

Well B: An unraised well with metal cover surrounded by grasses

Well C: A raised well with metal cover

Well D: An unraised well with metal cover surrounded by grasses

Well E: A raised well with metal cover

Well F: A raised well with metal cover constructed closed to a generator house

Well G: A raised well with metal cover

Well H: An unraised well with metal cover with an untidy environment

Well I: A raised well with metal cover

Well J: A raised well with metal cover

BIOGRAPHY

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