WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Bacteriological Assessment of Certain Streams and Boreholes in Some Selected Villages in Ekureku, Abi Local Government Area of Cross River State. Southern Nigeria.

***Enya Vincent I, Ofem Uduak L, Ogilagwu Sunday O. Ebong Ruth I, Eghong Clifford U.***

**Cross River State College Of Nursing, Itigidi**

**ABSTRACT**

Sustainable access to safe drinking water remains a global concern as many people in the world today still consume water from untreated and unimproved sources. The major concern about this study was the cholera outbreak which ravaged the entire Villages of Ekureku community in Abi Local Government Area of Central Cross River State, Southern Nigeria. In December, 2022, the second episode of the acute pandemic of the cholera outbreak struck the entire Villages of Ekureku community to the end March, 2023. History has it that a similar case occurred sometimes in 1993 and a lot of lives were lost. This study was carried out to evaluate the quality of eight (8) streams and two (2) boreholes (underground water) sources popularly used by the various villages in Ekureku community to access the suitability for drinking, domestic and industrial purposes. Water quality parameters analyzed include: Total bacteria counts (TBC) and faecal coliform counts.Several methods employed for these analyses include: various Bacteriological media. The results show that the total bacteria counts ranging from 55 CFU/ml in ST1 to $1.6 \times 10^2$CFU/ml in ST8. While BH1 has TBC of 16 CFU/ml and not detected in BH2. Faecal coliform counts ranged from 18/ml in BH1 to $60.5 \times 10^3$/ml in ST8, and not also detected in BH2.The study results show that, water samples from the streams contain a lot of microorganisms. The bacteria isolate from the water sample (streams) include *Escherichia coli, Proteus Vulgaris, Pseudomonas Spp, Shigella Dysenterae, Krebsiella Pneumoniae, Salmonella Spp, Streptococcus Aureus, Vibro Cholerae, Citrobacter Freundii, Bacillus Spp.*

## INTRODUCTION

The demand for water for human consumption, domestic and industriall purposes has grown considerably over the years and the supply by water board is grossly inadequate. Some people have therefore sort of other alternatives of water supply. This sorting to construction of boreholes (underground water) and the excessive use of surface water . Since streams, and boreholes are for human consumption, there is need for this water to be assessed for quality and to know how extend a particular stream or borehole is good for human consumption, domestic and industrial uses. Though, the borehole is significantly protected from surface pollutants as the earth media is composed of different surface layers as a natural filters. Supply of drinking water should be as pleasant as circumstances permit, coolness, absence of microorganisms, turbidity, colour and any other disagreeable taste or smell are of the utmost importance in public supply of drinking water. The construction situation, operation and its distribution system must be such as exclude any possible pollution of the water (WHO, 2011). Water provides energy in form of hydro electricity and certain countries like Nigeria are nearly 97% dependent on hydro power for their electricity production. Even for thermal and nuclear power station. Substantial amount of cooling water is necessary to dissipate heat. Industry cannot function without water, and water is invariably the focal point for many types of reactions and recreations. (Lohair & Thanh, 1978) The study of environmental water pollution in particular has therefore been of considerable importance not only to water analytical chemist, but also to engineers, hydrologist, toxicologist and pathologist since most of these determinants pose danger threat to man's life including other living organisms. Thus, it is very essential to analyze any water pose to human consumption, either to increase the need parameters or reduce to the required dose to avoid endangering the consumers and render it aesthetically suitable. Unlike oil and most other strategic resources, fresh water has no substitute in most of its uses. It is essential for growing food crops, manufacturing goods and safeguarding human health. Therefore, the development of groundwater or surface water constitute a viable supplement to the earth concrete dam. Fresh water is suitable for human, industrial and domestic uses.

## MATERIALS AND METHODS

Experimental research approach was employed in this study. Various bacteriological media and analyses were used for all the samples. The data for this research study were obtained from samples collected from eight selected (8) streams from different villages in Ekureku community and two (2) boreholes also from different locations as shown in table 1 for the purpose of this study and for easy characterization. The sampling points are given designations as ST and BH, meaning streams and boreholes respectively presented in table 2. The monitoring was carried out during rainy season.

**Samples collection**

Streams and boreholes were collected in sterile bottles, properly corked to avoid incoming air into the bottles containing the samples and transported in ice pack to the laboratory within twelve hours from the time of collection. The sampling site were randomly selected within Ekureku villages, Abi Local Government Area. The Cross River State Water Board (CRSWB) laboratory was used for this Analysis.

**Study area description**

The study area is Ekureku community in Abi Local Government Area of Central Cross River State, Southern Nigeria. Geographically, it is situated in the Northern part of Abi. It occupies a land mass of 262.971Km². The area is in the Central Cross River State of Nigeria. Two seasons by fluctuation of precipitation predominate in the area are dry and wet seasons from April to October (wet season) and November to March (dry season). The area is characterized by humid tropical climate (high temperature, humidity and precipitation).

**Population of the study area**

The study area Ekureku in Abi Local Government Area is one of the oldest community in Abi local government area in Cross River State. The area though, due to her large land mass, it is not densely populated but witness a serious population explosion, industrial and agricultural growth. It has 32,416 people as at (2022) extrapolated from 2006 National population census. The population rate and the agricultural activities directly deteriorate the quality of water resources.

**Selection of sites for sampling**

Ekureku community comprises of many major villages among which are Akarefor, Akarefor-Ezeke, Anong-Letafor, Anong- Likpoh, Ngarabe, Agbara, Ekureku-be, Etikwe, Akpoha, Egboronyi, Eminekpon, Itigevev and Gbagolo, Due to the geographical location of many of the villages, many streams in some of these villages do not survive the dry season and as such, samples were not collected in these areas. Samples were only collected in the areas that have good representative or average characteristics of other sources. However, certain criteria were applied in selecting sample sites as follows:

i. Sampling sites were selected such that the samples taken are representative of the different sources from which water enters in the streams.

Ii. Sampling sites were considered in such a way to take account of the number of inhabitants served by each source.

III. Sampling sites were of more interest considering the Industrial activities in the locations

TABLE 1: Showing sample locations including source and designation in Abi Local Government Area Cross River State, Nigeria.

| S/N | VILLAGE | LOCATION | SOURCE | SAMPLES CODE |
|---|---|---|---|---|
| 1 | Akarefor | Iwasu | Stream | ST1 |
| 2 | Agbara | Likpor | Stream | ST2 |
| 3 | Agbara | Ekorloba | Stream | ST3 |
| 4 | Anong-Likpoh | Edidor | Stream | ST4 |
| 5 | Ekureku-be | Esseng | Stream | ST5 |
| 6 | Agbara | Lehanghawe | Stream | ST6 |
| 7 | Eminekpon | Ebagili | Stream | ST7 |
| 8 | Ngarabe | Ikolo | Stream | ST8 |
| 9 | Agbara | St. Benedict | Borehole | BH1 |
| 10 | Akpoha | Akpoha | Borehole | BH2 |

**Sampling size**

The maximum sampling size varies widely depending on the range of variation to be considered and the analytical methods to be employed. Here, per 100mls were considered tb be maximum sample size, (WHO, 2017).

**Sampling procedure:**

Sampling from boreholes: Several procedures were followed to ensure a more precise results.

**Sample from Boreholes**

I. The tap outlet was thoroughly clean by wiping the outlet with clean cloth to remove any dirt attachment that could cause contamination of the water quality.

Ii. The tap was turn on to maximum and allowed to run for 1-2 minutes and then turn off.

III. It was sterilized for 1-2 minutes with a flame (from cigarette lighter).

iv. The water was then turn on and allowed to run at medium rate for 1-2 minutes.

v. The bottles were immediately filled with the sample, taken care to prevent entry of dust and air.

**Samples from streams:**

i. The sample cups and bottles were sterilized.

ii. The water samples were filled into the bottles using the sample cups.

III. The samples after collection are packed in a cooler containing ice bags.

iv. The samples were then taken to the laboratory within the shortest time.

## DATA ANALYSIS

**Inoculation**

**Method:** The membrane filtration method was used in this research work.

**Materials and reagents:** Water samples, sterilized 100ml measuring cylinders, sterilized forceps membrane filter papers (diameter 0.050um pore size, equiv. 0.45mm), pressure filtration apparatus comprising of upper and lower components, vacuum pump and sterile prepared agar media and alcohol.

The components of the filtration unit were accordingly sterilized. The autoclave particularly the upper and the lower components of the tunnel. A pair of forceps, four 100ml measuring cylinders were sterilized along side. The vacuum pump was connected to the special conical flask with a rubber tube. The upper component of the unit was replaced and clamped to make tight. 100ml of the sample water was measure in the sterilized measuring cylinder and poured in the vessel of the upper component of the unit. The vacuum pump was powered to activate filtration by suction pressure through the membrane filter. The pair of forceps was mobbed with alcohol and used to lift the membrane filter from filtration unit on the sterile agar surface. The filter trapped all microbes present in the water sample.

**Incubation/ BACTERIA GROWTH Enumeration**

Culture were incubated aerobically at 40°C for 24 hours. After this period, plates were pulled out of the incubator and the growth estimated by numerically counting the colonies and the number express as colony forming unit (CFU) per 100ml of water sample.

**Isolation of total coliform**

Membrane filtration technique was used, the agar was dissolved according to specification and autoclave at 121°C for 15 minutes. It was then dispensed aseptically and allowed to set. About 100ml of the sample was filter through the membrane filter which has been placed on the filter bed. The filter was removed and placed on agar plate. It was incubated at 37°C for 24 hours. The number of pink colonies were enumerated.

**Isolation of *Escherichia coli***

The ***Escherichia coli* (*E. Coli*)** were made on separate volume of water 100ml each. The water samples were filtered through sterilized membrane filters. The filters were then placed on an absorbent pad saturated with MacConkey agar, the sterile plate containing the pad and filter were incubated at 45°C for 24 hours, yellow colonies were isolate and counted as ***E. Coli***

**Isolation of *faecal streptococci***

Filter with pore size of about 0.05um was removed aseptically from the membrane filtration apparatus and placed on the culture plate with the blood agar, the plate were labelled and incubated at 37°C for 24 hours. All red colonies were isolated and counted as ***faecal streptococci***.

## CHARACTERIZATION AND IDENTIFICATION OF BACTERIA ISOLATES

All emergent colonies were noted and isolated in sub-cultures on the relevant differential and selective media to enhance characterization. The isolate were characterized and identified as listed in table 2.

**Gram stain**

The procedure grouped bacteria into gram positive or gram negative. A smear of the isolated colony was made on a glide heat fixed using burnsen flame. A few drops of crystal violet solution was added into the smear for 1 minute and washed off with water for 2 seconds, gram iodine was immediately applied then washed off with water. Finally, safranin, a counter stain was applied for 30 seconds and washed off with sterile water and dried, the stain smear was observed under the microscope using oil immersion objective lens. Organisms with violet or purple colour were termed gram positive while those with pink or red colour were termed gram negative.

**MOTILITY TEST**

The test is based on the fact that bacteria posses flagella and can move in this test. On a clean glass slide, a square of ridge was made with petroleum jelly. A drop of the broth of test organism was placed on clean cover slip. The slide was inverted to enable the jelly contact with the edge of the cover slip. The slip get sucked to the jelly on the slide, this was inverted to let the drop of broth hang on the under side of the cover slip on examination under microscope (x40 objective lens) motile organisms can be observed moving across the microscope field (locomotion), non motile will only shake or carried along by current on the medium.

**Citrate test**

Simon's citration slants were inoculated with isolates by stretching the slants surface. Incubation was done at 40°C for 24 hours. Citrate positive slants changed from green colour to bright blue. However, slow citrate utilization were incubated further. 38g of Simon's agar was weighed into 1 litre of distilled water. The mixture was boiled to dissolve. Media was sterilized in the autoclave at 121°C for 15 minutes. Titanium was dispersed into Mecartney bottles for slants.

**Methyl Red/Voges Proskauer test**

This test involves the preparation of peptone water, pipetted into test tubes and autoclave at 121°C for 15 minutes. It was later removed and allowed to cool for the inoculation of organisms to get settled. After that, the test tubes were incubated for 5 days at 37°C. At the end incubation, the culture was divided into half, the first half for methyl red test and the second half for the Voges Proskauer test.

**Methyl Red test**

(a) 0.5ml or 5 drops of 0.4% methyl red solution was added. A change in colour medium to red or pink indicating methyl red positive, that is, acid has been produced by the organisms. If yellow or no colour change indicates negative reaction.

(b) **Voges Proskauer test**

1ml of creative solution was added followed by a drop of sodium hydroxide solution. A reddish brown colour of the test indicates positive while yellow colour indicates no reaction.

**Coagulase test**

**Method:.** Slide method

**Procedure:** Two drops of plasma were placed on a clean grease free glass slide. A small portion of the test colony was emulsified in the plasma. Formation of Coagulates (Clumps) indicates a positive result.

**Indole test**

**Method:** Kovac's method

**Reagents/Apparatus:** P-dimethylamino benzaldehyde, Isoamyl alcohol, Conc. HCl, Kovac's reagent, Brown reagents bottle, peptone broth.

**Procedure:** P-dimethylamino benzaldehyde was dissolved in 75ml of amyl alcohol, then 25ml of Conc. HCl was added while stirring slowly. The solution was stored in a brown reagent bottle in the refrigerator. 2ml of 24 hours peptone broth of test organism, 0.5ml or 5 drops of Kovac's reagent was added and shake gently and observed. A red colouration which indicates a positive result.

**Substrate fermentation**

(a) **Lactose:** Media used was Mcconkey Agar plate and starts. Test organism was inoculated and plate incubated at 40°C for 24 hours. Positive lactose fermentation is shown as pink colonies.

(b) **Manitol:** Manitol Agar inoculated with test organism. Incubated as in lactose above. Positive Manitol fermenter emerge as yellow colonies.

(c) **Glucose:** Slant of Kligler Iron Agar (GIK) were inoculated with organisms and incubated at 40°C for 24 hours. Pink colour shows alkaline while yellow colour shows acid production from glucose fermentation. No colour change indicates non-glucose fermentation. Blackening at the point of the streak shows hydrogen sulphide ($H_2S$) production. Negative slants were further incubated to allow late fermentation and the subsequent production of hydrogen sulphide.

**Urease test**

Christensen Urea Agar slants were inoculated with test organism by streaking on the surface of the slants. Incubation was done at 37°C for 24 hours. A change from light orange colour to magnetta was positive Urease while no colour change was negative.

**Oxidase test**

A freshly prepared tetra-methyl-P-phenylenediaminedihydrochloride was poured into a clean filter paper. A colony of the isolate organisms was streak into the filter paper. A positive result was shown by the appearance of deep colouration within 1-5 seconds.

**TABLE 2**

**CHARACTERIZATION AND IDENTIFICATION OF BACTERIA ISOLATES**

| **S/N** | **COLONIAL CODE** | **LAC** | **MANN** | **CIT** | **IND** | **URE** | **OXI** | MR | VP | $H_2S$ | **MOT-** | CAT | COA | CONFIRMED ORGANISM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **a** | **+** | **+** | **+** | **+** | - | + | - | **+** | **-** | + | + | NR | ***Escherichia Coli*** |
| **2** | **b** | **+** | **+** | **+** | **-** | + | - | + | **-** | **-** | + | NR | NR | ***Proteus vulgaris*** |
| **3** | **c** | **-** | **-** | **+** | **+** | - | + | + | **-** | **-** | + | + | NR | ***Pseudomonas Spp*** |
| **4** | **d** | **+** | **+** | **+** | **-** | - | - | + | **-** | **-** | - | + | - | ***Shigella Dysenterae*** |
| **5** | **e** | **+** | **+** | **-** | **-** | - | + | - | **+** | **-** | - | - | NR | ***Klebsiella pneumoniae*** |
| **6** | **f** | **-** | **+** | **+** | **+** | + | - | - | **-** | **+** | + | + | NR | ***Salmonella Spp*** |
| **7** | **g** | **-** | **+** | **+** | **NR** | NR | NR | NR | **NR** | **-** | - | + | - | ***Streptococcus Aureus*** |
| **8** | **h** | **+** | **+** | **+** | **-** | + | - | + | **-** | **+** | + | + | NR | ***Vibro Cholerae*** |
| **9** | **i** | **-** | **-** | **+** | **+** | NR | NR | NR | **NR** | **NR** | - | + | - | ***Bacillus Spp, Plasmodium*** |
| **10** | **j** | **+** | **+** | **+** | **+** | - | + | - | **+** | **+** | + | + | + | ***falciparum*** |
| | | **+** | **+** | **+** | **-** | + | + | + | **-** | **+** | + | + | + | ***Citrobacter Freundii*** |

**KEY:**

Lactose (LAC), Manitol (MANN), Citrate (CIT), Indole (IND), Urease (URE), Methyl Red (MR), Voges Proskauer (VP), Hydrogen sulphide ($H_2$), Oxidase (OXI), Motility (MOT), Coagulase (COA), Catalase (CAT), No reaction (NR), positive reaction (+), Negative reaction (-).

**TABLE 3. BACTERIA ISOLATED PER SAMPLE**

| SAMPLE CODE | SAMPLE LOCATION | BACTERIA ISOLATION |
|---|---|---|
| ST1 | IWASU | Streptococcus faecalis, Staphylococcus aureus, Vibro Cholerae, Pseudomonas Spp, Proteus Vulgaris |
| ST2 | LIKPOR | Escherichia coli, Salmonella Spp, Shegella Dysenterae, Krebsiella Pneumoniae |
| ST3 | EKORLOBA | Bacillus Spp, Enterobacter Aerogenosa |
| ST4 | EDIDOR | Staphylococcus Spp, Proteus vulgaris, Pseudomonas Aerogenosa |
| ST5 | ESSENG | Bacillus Spp, Salmonella Typhi, Salmonella Paratyphi, Escherichia coli |
| ST6 | LEHANGHAWE | Enterobacter Freundii, Proteus Vulgaris, Vibro Cholerae, Salmonella Typhi, Escherichia coli |
| ST7 | EBAGILI | Shegella Dysenterae, Streptococcus Aureus, Vibro Cholerae, Bacillus Spp |
| ST8 | IKOLO | Klebsiella pneumoniae, Salmonella Typhi, Plasmodium falciparum, |
| BH1 | ST. BENEDICT | Escherichia coli |
| BH2 | AKPOHA | Not detected |

## CONCLUSION

The study revealed that, the streams in the area recorded high bacteria counts ranging from 55CFU/ml to $1.6 \times 10^2$CFU/ml in some streams. High faecal coliform counts were also recorded, with count ranging from 18CFU/ml to 60.5CFU/ml exception of BH2 which no bacteria was recorded and no faecal coliform was also detected. The water in the area are not good for human consumption. The most probable number (MBN) analysis results of coliform/100ml in the area given clear indication of poor quality of the various streams exception of the Boreholes especially the Borehole located at the Akpoha Community (BH2). This is concluded that the Boreholes water are of high quality than the streams, since in the Boreholes, bacteriological analysis revealed less or no bacteria isolates and faecal coliform counts. The bacteria isolates in the streams were all analyzed and the results revealed, which were higher than WHO standard for CFU per 100ml recommend for water sample. The various bacteria growth in the streams, for example, Iwasu stream ST1 has bacteria growth include ***Streptococcus faecalis***, ***Vibro Cholerae*** , ***Pseudomonas Spp, Proteus Vulgaris, Staphylococcus aureus.*** Other streams also have comparative numbers of bacteria growth as revealed from the bacteriological analysis. See table 3. The Cholera outbreak which ravaged the community, struck on 18th December, 2022 to the end of March, 2023. many lives were lost during this period. This is attributed to the poor quality of water available for the community.

## RECOMMENDATION

The surface water is extremely poor for human consumption, this is because the total coliform counts and the Faecal coliform units were higher than WHO standard for drinking water. Unwanted materials be recycled more often instead of being dumped into water bodies, as this may lead to sewage, which enhance the growth of various species of microorganisms in the water.

The streams water should be thoroughly boiled before drinking. This is to reduce the bacterial loads or kill the microorganisms present in the water in order not to cause harm to the body.

The boreholes water is safer as compared to the surface water, this is because, the underground has impervious layers of natural filters and no waste material is allowed to penetrate into the water body. Therefore, the boreholes are better for drinking and other domestic purposes.

The Government should construct as many Boreholes as possible, or at least, each village should be given a standard borehole big enough to distribute water round the outlets.

Finally, routine assessment of bacteriological analysis of water be carried out frequently on seasonal basis as means of controlling the hygienic safety of streams and boreholes of supply of water.

**REFERENCES**

Kassie G.G et. al. (2017): Bacteriological Quality of Drinking Water from source to point use Among Rural Communities of Farts Warda in Northwest Ethiopia. Afr J. Microbiol. Res.11(26):1069-74.

Bekuretsion H. et. al. (2018): Bacteriological Assessment of Drinking Water from Hand-Pump Fitted Borehole Source. In Kola Tembien Central Tigrai and Northern Ethiopia. Ethiopian J. Water Supply. 68(8); 190-9.

WHO (2011). Guidelines for Drinking Water Quality. Geneva, Vol.1 WHO 4th Ed.

USAID Ethiopian Water [Cited, 2015 November 23]. Available from wwwusaidethiopian.water-and-sanition.gov

Miner C.A. et. al. (2016). Physical and Microbiological Quality of Water Sources in Gwafan Community Plateau State, Nigeria. Pyrex Res Environ Stud.3(1); 001-6

WHO (1993). Drinking Water Quality Guidelines. (4 Vols.) Geneva: World Health Organization.

Saleh, H.N. et Al. (2019): Carcinogenic and non-carcinogenic Risk Assessment of Heavy Metals in Underground Water (Boreholes) in Neyshabur Plain, Iran, Biol, Trace Elem, Res170, 251-261

Agnesi, A., Tibyangye, J., Tamale, A., Agwu, E.& Amongi, C. (2019): Contamination Potentials of Boreholes Water Handling and Storage Practices in Kirundo, Subcounty Kisoro District, Uganda, J. Environ. Public Health Article ID7933193, 8 pages.

WHO(2021): Guidelines on Recreational Water Quality. Vol.1 Coastal and Fresh Waters. Geneva. World Health Organization.

APHA (1975): Standard Method for the Examination of Water and Waste Water., 14th ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington, DC

Bartram, J.B. (1990): Drinking Water Supply Surveillance. Robens Institute, University of Survey, Guildford.

Chapman, D. ed. (1996): Water Quality Assessment. A Guide to the Use of Biota, Sediment and Water in Environmental Monitoring. 2nd ed. Chapman and Hall, London.

Edet, A. E , Uka, N. K., Offiong, O.E. (2001): Chemistry and Discharge Characteristics of Odukpani Junction Springs, Calabar Flank South-Eastern Nigeria. Environment Geology. 40, 1214-1215

UNGA (2010): Resolution 64/292; The Human Right to Water and Sanitation. In: 108th plenary Meeting, 28th July, 2010. United Nation General Assembly).

Eja, M.E. (2001): Water Pollution and Sanitation for Developing Countries. Seaprint, Calabar.

Cotruvo, J. A. et al (2010): Desalination Technology, Health and Environmental Impacts, Boca Raton, FL, CRC Press.

Krishnan, K., Carrier, R. (2013): The use Exposure Source Allocation Factor in the Risk Assessment of Drinking Water Contaminants. Journal of Toxicology and Environmental Health, Part B. Critical Reviews, 16(1); 39-51

Etu-Efoetor, J. O. (1998): Hydro-chemical Analysis of surface and Ground Water of Gwagwalada Area of Central Nigeria. Global Journal of Pure and Applied Science. 4(2); 153-159

APHA, AWWA, WEF. (2017) 7110C: Coprecipitation Method for Gross Alpha Radioactivity in Drinking Water. In: Baird RB, Eaton, AD, Rice EW, ed. Standard Methods for the Examination of Water and Wastewater, 23rd ed. Denver American Public Health Association, American Water Works Association, Water Environment Federation.

ISO (2017): Standard ISO 9696 Water Quality-Measurement of Gross Alpha Activity in Non-saline Water, Thick Source Method. Geneva. International Organization for Standization.

Ford, T.E. (1993): Aquatic Microbiology, Cambridge. Man's Blackwell Scientific Publication Inc.

Esu, E.O & Amah, E.A. (1999): Physico-chemical and Bacteriological Quality of Natural Waters in part of Akwa Ibom and Cross River States, Nigeria. Global Journal of Pure and Applied Science 5(4); 525-532.

Richard, J., Wagman, M.D. F.A.C.P.(2000): The New Complete Medical and Health Encyclopedia. (3); Freguson Publishing Company, Chicago.