A Field Test for Assessment of Feacal Contamination of Potable Water in the Kumasi Metroplis of Ghana

Doreen Amponsah, Godfred Etsey Sebiawu Wa Polytechnic, Wa, Ghana.

Abstract: The Salmonella Escherichia coli medium (SEM) was developed to detect the faecal contamination in portable water. This method is very simple and rapid. It is capable of detecting the presence of salmonella species and Escherichia coli in water. Results are obtained within 18-24 hours. The SEM method agrees 99% with the traditional conventional most probable number. In areas devoid of laboratory, this method can be used and it proves very efficient in the field. Portable water is unsafe if the water turns black in the test tube containing the SEM paper strip and red ring is observed after addition of Kovac reagent or the portable water is safe if the water does not turn black after 24hours in the test tube. Samples of well, borehole and tap water in the Kumasi metropolis were analysed.

Keywords: Portable water, Feacal contamination, Salmonella, Escherichia coli. Coliforms, Kovac Reagent, Laboratory

I. INTRODUCTION

It is well established fact that a large number of infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta particularly faeces [1]. Outbreaks of water borne diseases continue to occur throughout the world but especially serious in developing countries [2], [3]. The World Health Organization attributed 4.0% of all deaths and 5.7% of the global disease burden to water-related illnesses, which stemmed from poor water quality, hygiene and sanitation [4].

The provision of good quality water which is odourless, colourless, tasteless, and free from faecal pollution is one of the most important infrastructural problems facing many developing countries [5], [6]. According to the world health Organization in 1988, only 65 % have houses tap connections and additional 20 % have access to public taps whiles 32% lack portable water supply, but the ratio has improved since 1990, the number of people in rural areas using an unimproved water source in 2010 was still five times greater than in urban areas [7]. About 884 million people in the world still do not get their drinking-water from improved sources, almost all of them in developing regions. Sub-Saharan Africa accounts for over a third of that number, and is lagging behind in progress towards the MDG target, with only 60% of the population using improved sources of drinking-water despite an increase of 11 percentage points since 1990 [8].

In urban Ghana, most people enjoy good treated water with 95% of household receiving water directly in their homes and at community standpipes (GWCL, 1998). As a

result of increasing urbanization, a number of peri-urban communities have developed with city authorities unable to cope with the infrastructural development in these areas. These communities have no access to treated pipe-borne water and often rely on boreholes, streams and wells for water supply. Well water is partially purified due to the natural filtration of the water peculating through the layers of the soil and rocks [9]. This does not rule out the presence of microorganisms that travel rapidly through the fine grained fractured sub soils underlying the soil to the water table alongside rainwater. Studies have shown that a large fraction of coliform contaminants in drinking water distribution systems is likely be associated with organic and inorganic deposits and biofilms [10], [11], [12].

Fecal contamination often results from the discharge of raw sewage into natural waters, a method of sewage disposal common in developing countries, and even in more advanced countries like China, India, and Iran [13]. Waterborne disease may be transmitted by consumption of polluted drinking water, by immersion in recreational water or by contact through skin or inhalation. The health effects caused by microorganism have been reviewed extensively and well documented [14], [15], [16], [17].

The environment play significant role in the transmission of many diseases, though it is relatively uncommon in countries which have a good standard of hygiene [18]. The reported prevalence of infection among patients with pancreatic necrosis is 40-60% with E.coli and Salmonellae been commonest [19].

The purpose of this research is to prepare a one-step absorbent pad that will determine the presence of both E.coli and Salmonella species in portable water.

A. Causes of Water Contamination

E.coli is actually a member of the family enterobacteria. It is a gram-negative growing aerobically and anaerobically at an optimum temperature of 37 °C and readily killed above 55°C. It is commonly found in the intestine of animals including birds. E.coli is an example of faecal coliform and they occur almost entirely in faeces [23]. Chlorination is used to kill all coliform. The presence of coliforms even in small quantities after chlorination means the process has failed. Other indicators of water quality are streptocci and clustridici. E.coli is also the commonly cause of urinary tract infections and gram-negative rod sepsis. It is the most

abundant facultative anaerobe in colon and faeces. E.coli ferments lactose a property that distinguishes it from the two major intestinal pathogens shigella and salmonella. It has three antigens that are used to identify the organism in epidemiologic investigation [20]. E.coli comes from human and animal wastes. During rainfalls, snow melts or other types of precipitation, E.coli may be washed into rivers, lakes, or groundwater. When these waters are used as sources of drinking water and the water is not treated or inadequately treated, E.coli may end up in drinking water.

Faecal coliforms are bacteria that are associated with human or animal wastes usually live in human or animal intestinal tracts. Bacterial indicator organisms such as faecal coliforms have been used to test water samples for faecal pollution, but such indicators do not provide specific information on the specific source of pollution. These bacteria may be found in a variety of warm- blooded animals and are not unique to human intestinal flora.

Water is fit and portable for human consumption so long as human pathogens are absent. Pathogens can be introduced into natural water system in a variety of ways [24].

B. Method of Detection

There are many methods that are used to determine the presence of salmonella but do not detect the presence of the coliform organism itself. A desirable simplified test which is capable of bringing out both reactions in a single test tube procedure as a mere confirmatory screening test is the salmonella E.coli medium. It is formulated and used to determine or test for the faecal contamination in portable water. It is very simple, rapid, sensitive and inexpensive:

The SEM method is a one-step method using the test tube procedure of simultaneous detection of indole that represents coliform bacteria and hydrogen sulphide to indicate the presence of salmonella species. The SEM method compared to the standard traditional method, the most probable number for detection of coliform in water was found to be more accurate, economical and reliable.

II. STUDY AREA

The Kumasi Metropolis is the regional capital of the Ashanti Region of Ghana. The Kumasi Metropolis is located between latitudes 6.35°N to 6.40°N and longitudes 1.30°W to 1.35°W. The Metropolis covers a land area of approximately 254km² and approximately ten (10) kilometers in radius. There are 103 communities. Currently, the Kumasi Metropolis is ranked second in Ghana in terms of land area, population size, social life and economic activity to the national capital Accra.

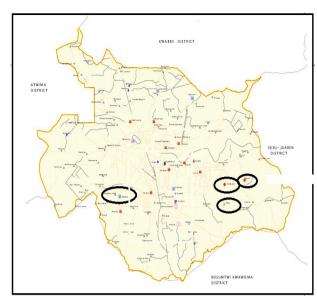


Fig.1. Shows Map of Kumasi Metropolis.

Sample sites include Ayeduasi, Boadi, Kwadaso and Kotei as circled in the Map in Fig.1.

III. METHOD AND MATERIALS

A. Apparatus

Glass test tubes, conical flask, absorbent pad, Cotton plugs.

B. Preparation of the Salmonella E.Coli Medium.

About 10% bacterotryphone, 5% yeast extracts, 5% sodium formate, 55 sodium chloride, 0.25% DL-tryptophan, 0.5% sodium thiosulphate, 0.755 ferric ammonium citrate, 0.05% sodium lauryl sulphate was added to 100ml distilled water and prepared with a final pH of 6.8. It had a colour of pale brown. Half of the absorbent pad was soaked by 0.5ml of the salmonella E.coli medium and placed in a glass test tube. The pH of the SEM was 6.8 and it was determined by using a pH meter in the laboratory.

C. Experimental Procedure

 $0.5 \mathrm{ml}$ of the salmonella E.coli medium was added to half of absorbent pad and placed in a glass test tube with non-absorbent cotton plugs and autoclaved at $121^{0}\mathrm{Cfor}$ 15minutes. It was followed by air drying in an oven at $50^{0}\mathrm{C}$. The SEM tubes were stored in a cool dry place.

A 10ml portion of water sample to be tested was added to the SEM tubes and incubated at room temperature. Tubes were noted positive within 16-24 hours and were graded as unsatisfactory if the water turned black but satisfactory if the water does not turn black.

D. Kovac reagent

Kovac reagent was prepared by mixing 5g of p-dimethylamino-benzaldehyde, 75ml amyl alcohol and 25ml concentrated hydrochloric acid. This reagent was used to confirm the presence of E.coli.

E. Sterilization

Equipment and materials that needed sterilization were given such treatment. Autoclaving is used as method of sterilization.

F. Autoclaving

The media in the glass ware were plugged with non-absorbent cotton wool prior to sterilization. This was done in the autoclave at a temperature of 121^{0} C for 15mins.

IV. RESULTS AND DISSCUSION.

Table 1: Comparism of SEM test and conventional MPN method for detection of bacterial contamination in different sources of water at the Kumasi metropolis.

Sample/ml	10	1.0	10-1	10-2	10-3	10-4	No of bacteria
							per 100ml
Comple Aurine	2	1	0	0	0	0	sample
Sample A using SEM		1				0	150000
Sample A using MPN	2	1	0	0	0	0	150000
Sample B using SEM	2	1	0	0	0	0	150000
Sample B using MPN	3	1	0	0	0	0	4300000
Sample C using SEM	3	3	3	1	0	0	460000
Sample C using MPN	3	3	3	0	0	0	230000
Sample D using SEM	3	3	2	0	0	0	90000
Sample D using MPN	3	2	2	0	0	0	210000
Sample E using SEM	3	3	3	0	0	0	230000
Sample E using MPN	2	3	2	0	0	0	200000
Sample F using SEM	2	1	0	0	0	0	150000
Sample F using MPN	2	1	0	0	0	0	150000
Sample G using SEM	3	3	2	1	0	0	40000
Sample G using MPN	3	3	3	1	0	0	460000
Sample H using SEM	3	3	1	0	0	0	430000
Sample H using MPN	3	1	0	0	0	0	430000
Sample I using SEM	3	3	3	0	0	0	230000
Sample I using MPN	3	3	3	0	0	0	230000
Sample J using SEM	1	0	0	0	0	0	40000
Sample J using MPN	1	0	0	0	0	0	40000

Sample I using SEM	2	1	0	0	0	0	150000
Sample I using MPN	2	1	0	0	0	0	150000
Sample K using SEM	3	3	2	2	0	0	210000
Sample K using MPN	3	3	3	1	0	0	430000
Sample L using SEM	3	3	1	1	0	0	430000
Sample L using MPN	3	3	1	1	0	0	430000
Sample M using SEM	1	1	2	0	0	0	90000
Sample M using MPN	1	1	2	0	0	0	90000
Sample N using SEM	1	1	0	0	0	0	40000
Sample N using MPN	1	1	0	0	0	0	40000
Sample P using SEM	0	0	0	0	0	0	Not detected
Sample P using MPN	0	0	0	0	0	0	Not detected
Sample Q using SEM	0	0	0	0	0	0	Not detected
Sample Q using MPN	0	0	0	0	0	0	Not detected
Sample R using MPN	0	0	0	0	0	0	Not detected
Sample R using SEM	0	0	0	0	0	0	Not detected
Sample 5 using MPN	0	0	0	0	0	0	Not detected
Sample 5 using SEM	0	0	0	0	0	0	Not detected
Sample 1 using MPN	0	0	0	0	0	0	Not detected
Sample 1 using SEM	0	0	0	0	0	0	Not detected
Sample 2 using MPN	0	0	0	0	0	0	Not detected
Sample 2 using SEM	0	0	0	0	0	0	Not detected
Sample 3 using MPN	0	0	0	0	0	0	Not detected
Sample 3 using SEM	0	0	0	0	0	0	Not detected
Sample 4 using MPN	3	3	3	1	0	0	460000
Sample 4 using SEM	3	3	3	0	0	0	230000
Sample 5 using MPN	3	3	3	0	0	0	230000
Sample 5 using SEM	3	3	3	0	0	0	230000
Sample 6 using MPN	2	1	0	0	0	0	150000
	_						

Vol.	3	Issue	7	Inly	_	2014	1
VOI.	J	issue	/,	July	-	2014	1

Sample 7 using SEM	2	1	0	0	0	0	150000
Sample 7 using MPN	1	1	0	0	0	0	70000
Sample 8 using SEM	1	1	0	0	0	0	70000
Sample 8 using MPN	0	0	0	0	0	0	Not detected
Sample 9 using SEM	0	0	0	0	0	0	Not detected
Sample 9 using MPN	0	0	0	0	0	0	Not detected
Sample 10 using SEM	0	0	0	0	0	0	Not detected
Sample 10 using MPN	0	0	0	0	0	0	Not detected

DISSCUSION

From the table of result, it can be observed that the number of bacteria per 100ml Sample using Mackonkey broth was almost the same as the number of bacteria per 100ml sample using SEM but this may be due to probability, where the mean of these experimental results obtained from the laboratory for these method gave 99% agreement with the result obtained by N. jothikumas et al. The SEM medium is actually capable of detecting both salmonella species and Escherichia coliforms. It was also a selective medium that permit the growth of Specific micro-organism and also sensitive for permitting the growth of the Salmonella and Escherichin coliforms. There are a few other methods that detect either salmonella or Echenchin coliform but the SEM medium works for both [32].

In general, it is evident that the SEM test is more consistent than the Conventional MPN technique. Escherichia coli and other enteric bacteria such as Proteeus, citrobacter deversis and Edwardsiella do produce indole but the use of tryphophan as ingredient distinguished Escherichia coli from the other enteric bacteria. There is no correlation between the numbers of E.Coli and risk of illness recurring in a given sample. The SEM medium contains ingredients such as sodium lauryl sulphate which retards the growth of gram positive bacteria [33]. Sodium formate resuscitates injured bacteria in the medium yeast extract stimulated the salmonella growth rate and tryptophan which is hydrolyzed to indole [37]. The production of indole from tryphtophan is useful in distinguishing Escherrichia coli from other enteric bacteria [39]. The medium also gives multiple reaction namely indole and hydrogen sulphate in less than 24 hours.

The SEM paper (medium) autoclaved at 121°Cfor 15minutes is actually done for killing all micro-organism and viruses in the material. This process is known as sterilization. This requirement is very important because changing the conditions of growth can sometimes revive micro-organism and viruses in the material. This process is known as sterilization. This requirement is very important because changing the conditions of growth can sometimes revive

micro-organism and viruses that have apparently been killed. Autoclaving is the most simple and consistently effective means of sterilization.

Ground water can be contaminated by improper disposal of solid and liquid waste. Sewage may contaminate surface water such as well and borehole but given because of poor construction of well which may enable surface run off to enter the well. The Depth of the well can also be a factor.

VI. CONCLUSION

The SEM method which was used for the detection to Salmonella E.Coli bacteria is simple for use in the field. A positive test was observed visually with a change in colour at blackening of the sample confirming the presence of Salmonella contamination and upon addition of few drops of Kovac regents. There was a red ring formation indicating positive coliforms and clear indication of E.Coli. The results were obtained within 18hours. The SEM method involved no expensive equipment and no tedious pipetting [29].

VII. RECOMMENDATION

It is recommended that further work be carried out to develop a simple method to purify the potable water after it has been detected by SEM medium to be unsafe. The SEM paper should be made in a sachet form to enable easy carriage.

REFERENCES

- WHO. Guidelines for drinking water quality. Geneva: World Health Organization. Vol. 1: pp1-29. 1997.
- [2] K. A. Reynolds, K. D. Mena, C. P. Gerba. Risk of water-borne illness via drinking water in the United States. Rev Environ Contam Toxicol. 192: 117-158. 2007.
- [3] A. Q. Jones, S. E Majowicz, V. L. Edge, M. K. Thomas, L. Mac-Dougall, M. Fyfe, S. Atashband, S. J. Kovacs: Drinking water consumption patterns in British Columbia: an investigation of associations with demographic fac-tors and acute gastrointestinal illness. Sci Total Envi-ron. 388: 54-65.2007.
- [4] A. Pruss, D. Kay, L. Fewtrell, J. Bartram. Estimating the burden of disease from water, sanitation, and hygiene at a global level. Environ. Health Perspect. PubMed: 12003760. 110 (5):537–542.. 2002.
- [5] WHO. Report on The Global Burden of Disease concept. 2002a.
- [6] WHO. Report on The Global Burden of Disease concept. 2002b.
- [7] WHO/UNICEF Report on Progress on Drinking and Sanitation. Update 2012. Retrieved 10 July 2014, from http://water.org/water-crisis/water-facts/water/
- [8] WHO/UNICEF Report on Progress on Drinking and Sanitation. Update 2010. Retrieved 10 July 2014, from http://water.org/water-crisis/water-facts/water/
- [9] L. J. Prescott, J. Harley, and D. Klein. Microbiology. 3rd ed. WCB Publishers. Chicago, IL. Pp. 811-877. 1996.
- [10] A. K. Camper. (1994) Coliform regrowth and biofilm accumulation in drinking water systems: a review. In Biofouling ed. Geesey, G.G., Lewandowski, Z. and Flemming, H.C. pp. 91–105. Boca Raton, FL: CRC Press. 1994.
- [11] S. Fass, M. L. Dincher, D.J. Reasoner, D. Gatel and J.C. Block. Fate of Escherichia coli experimentally injected in a drinking water distribution pilot system. Water Research 30, 2215–2221.1996.
- [12] S.L. Percival, J.T. Walker, and P.R. Hunter. Microbiological Aspects of Biofilms and Drinking Water. Boca Raton, FL: CRC Press .2000.
- [13] G.M. Carr and J.P. Neary. Water Quality for Ecosystem and Human Health, 2nd Edition. United Nations Environment Programme Global Environment Monitoring System 2008. Retrieved 10 July 2014, from http://www.gemswater.org/publications/pdfs/water_quality_human_health.pdf
- [14] N.S. Galbraith, N.J. Barnelt and R. Stanwell-Smith. Water and Disease after Croydon. A reviewed of water associated diseases in the United Kingdom(1937-1980) Journal of Institution of Water and Environmental Management, 1, 7-21. 1987.
- [15] P.R. Hunter. Waterborne Disease. Epidemiology and Eology. John Wiley and Sons, Chichester, United Kingdom. 1997.
- [16] J. V. Dadswell. Microbiological quality of coastal waters and its health effects. Internat. Environ. Health Res. 3:32–46. 1993.

- [17] A. Prüss. A review of epidemiological studies from exposure to recreational water. International Journal of Epidemiology, 27: 1– 9 1998
- [18] H. C. Maguire, E. Holmes, J. Hollyer, J. E. M. Strangeways, P. Foster and R. E. Holliman, R.E. An outbreak of cryptosporidiosis in South London: what value the p value? Epidemiology and Infection 115, No. 2, 279-287. 1995.
- [19] Thomas Bollen, Marieke Hazewinkel and Robin Smithuis Pancreas -Acute Pancreatitis. Cambridge Journal of Epidimiology and Infection. 2008
- [20] Medical microbiology and immunology. Warren Levin son, Ernest Jawetz
- [21] J. A Clarke, Journal Microbiology, P.15, 1995.
- [22] R. E Mckinney, Microbiology for sanitary engineer Mac Grow Hill Book Company Inc, P. 152-152, 1962.
- [23] R. W. S. Harvey, A review: Principle of Salmonella isolation. Journal of applied Bacteriology, P.42, 105, 1996.
- [24] D. S Crowley, MJ Ryan, P.G Wall, Gastro- Enteritis in children P.82, 1997.
- [25] H. S Evans, P. Madden, C. Douglas, General outbreak of Infectious Intestinal Disease P. 165, 1998.
- [26] J. E Finch, J. Prince, M. Hawksworth, A bacteriological survey of the Domestic environment, Journal of Applied Bacteriology 357, 45, 1978.
- [27] A. Lipso, Infection dose of Salmonella, 1976.
- [28] O. G wheeler, D Sethi, J.M S. S Cowden, A study of infectious intestinal diseases p. 1046, 1999.
- [29] S. G Gerzof, P.A Banks, A. H Robbins, Early diagnosis of Pancreatic infection by computed tomography guide aspiration Gastroerology, P. 93, 1987.
- [30] A. P. Jones, J. Sellwood, Enteric Viruses in the aquatic environment. Journal of applied Microbiology, P 945-962.
- [31] A. Fennel, A single tube confirmatory test for E.Coli. Water treatment and examination, P. 13,4, 1972.
- [32] Nester, Robert, and nester, Microbiology for human perspective, P. 227.
- [33] M. Hutchinson, J.W Ridgeway, Microbiological aspects of drinking water supplies in aquatic microbiology, P. 179.
- [34] D. A. A Mossel Lancet, Microorganism Journal P. 184.
- [35] Cox, C. R, Operation and Control of water treatment processes, 15. 66.
- [36] E. B Parker, and John Son, Introduction to contaminant hydrogeology, p. 583, 1968.
- [37] J. Odlikornar, N. Subba, K. Rao, Madras Zonal Lab Chennai 600, 113, India P. 183.
- [38] D. M Paul. The Microbilogy of Water HMSO, P. 120-121, 1994.
- [39] Prescolt Harley Klien, Microbiology, 3rd Edition, P. 20-25, 1976.Lipschitz-Hankel type involving products of Bessel function Phil. Trans. Roy. Soc. London, vol. A247, pp. 529-551, April 1955.