Chapter 2

Review of Literature
REVIEW OF LITERATURE

The H₂S method has been extensively studied by a number of investigators in different parts of the world. Such studies included evaluations of the original method, studies on modifications of the method and field-testing, usually with side-by-side comparison to other water quality monitoring tests. In some of these comparative studies, the data was limited or have not been subjected to rigorous statistical analysis. However, the results of most studies suggested that the H₂S method detect fecal contamination in water with about the same frequency and magnitude as the traditional methods to which it was compared. Testing conditions and format, sample size, incubation temperature and incubation time influences test sensitivity. These conditions have differed among the different studies reported in the literature and it is difficult to make consistent comparison and draw overall conclusion. However when comparison with other methods for detecting fecal contamination was done, the H₂S method appeared to be more precise.

In the initial development of the H₂S test for drinking water quality monitoring, Manja et al. (1982) contributed a lot. The test was applied to various drinking water samples of several cities in India. Water samples containing 10 or more coliform bacteria by MPN were subjected to the new H₂S test using a 20-ml sample volume in a P-A format. On this basis positive H₂S tests were considered unsatisfactory. Since this was the original description of the H₂S test, several investigators have reported modifications of the test intended to improve its performance. Such modifications have included test medium, medium preparation (dried at elevated temperature, lyophilized, autoclaved only, etc.) sample volume (20 ml, 100 ml, etc.), paper use, paper type and paper size to which the medium was absorbed, incubation time and temperatures and test formats (presence-absence, quantitative MPN and membrane filter enumeration).
They applied the test to water samples having 10 or more total coliform MPN per 100-ml, scored the same number of samples suitable and unsuitable by both MPN coliform tests (10/100 ml = suitable; >10/100 ml = unsuitable) and the H₂S test (not black in 20 ml = suitable and black in 20 ml = unsuitable). When the water samples were divided into ranges of coliform concentrations, the H₂S test gave positive reactions for all samples with 41 or more coliforms per 100 ml, 25/34 H₂S positive for samples with 21-40 coliforms per 100 ml and 37/44 positive with 11-20 coliforms per 100 ml. H₂S positive-samples contained *Citrobacter freundii* (23 samples), *Salmonella* spp. (6 samples), *Proteus mirabilis* (2 samples), *Arizona* spp. (2 samples), *Klebsiella* spp. (1 sample) and *E. coli* (3 samples). Only one type of H₂S-producing organism was isolated from each separate sample and the methods of isolation were not specified. No tests were done for the presence or absence of other, environmental H₂S-producing bacteria. No specific tests were done to determine the presence of viral, bacterial or parasitic pathogens in the study, although *Salmonella* spp. was detected in some samples. Authors considered that the test was reliable, simple to perform and useful for screening purposes where resources, time, manpower and laboratory facilities are limited.

Moreover Sivavorvorn (1988) tested 705 samples from a variety of water samples in Thailand (shallow and deep wells, rainwater, pond water) by the original H₂S test and by coliform MPN. Based on agreement between a positive H₂S test and 10 MPN/100 ml as a coliform or fecal coliform positive test, the two tests agreed 85% and 88% of the time, respectively. It was concluded that the H₂S test could be used to screen water for fecal contamination in the field where laboratory facilities are limited.

After that Ratto et al. (1989) evaluated the original H₂S test at incubation temperatures of 22 and 35°C. It was compared with MPN, P-A total coliform (TC) and fecal coliform (FC) tests for 20 water samples from Lima, Peru. The frequency of positive (unsuitable) samples was similar but not identical for all tests: 9/20 by P-A, 9/20 by H₂S at 35°C, 6/20 by H₂S at 22°C, 8/20 by TC MPN and 6/20 by FC MPN. They concluded that the H₂S test was an equally or more sensitive test than
TC and FC tests and was an ideal procedure for water supplies where laboratory facilities do not exist.

Bukenya (1990) determined the validity and predictive value of the hydrogen sulphide-screening test for water quality compared to a standard multiple-tube technique. The hydrogen sulphide test was found to be highly sensitive and specific with a high predictive value and recommended as a screening test for water quality in developing countries where resources were limited especially in rural water supplies.

There are several modifications to the \( \text{H}_2\text{S} \) test that could be considered in an effort to make it more specific for organisms of fecal origin and to reduce the probability of organisms of non-fecal origin giving a positive response. These modifications fall into two main categories: modifications of the medium itself and modifications of the incubation conditions.

For more efficiency of the \( \text{H}_2\text{S} \) test, some modifications were done in performing the \( \text{H}_2\text{S} \) test. In 1994, Venkobachar and his coworkers developed a modified \( \text{H}_2\text{S} \) test that included cysteine in the medium and was used in MPN test with five 20-ml samples. The original \( \text{H}_2\text{S} \) test was compared with the modified medium containing cysteine using different water sources. Correlation analysis indicated that the cysteine-modified test was more sensitive and less time-consuming than the original test. The modified test reduced the test time from 23 to 17 hours and was more sensitive than the original \( \text{H}_2\text{S} \) test. It was well correlated with total coliform (89%) and fecal coliform (91%) tests when applied to 101 water samples. They concluded that \( \text{H}_2\text{S} \) test was simple, requiring little laboratory support and well suited for routine quality assessment of rural water sources.

Kaspar et al. (1992) evaluated a modified version of the original \( \text{H}_2\text{S} \) test (no tissue paper and lyophilizing, rather than heat drying of the medium) and applied it to 101 water samples in Paraguay. Using an improved preparation procedure, results were basically temperature independent in the range of
22-37°C. Results were correlated well with the presence of total coliform bacteria (96%, 28°C) and observed apparently false positive results in H₂S test. In ground waters, particularly those contaminated with human or animal wastes, fecal or otherwise, or those containing reduced sulfur from natural or anthropogenic sources, there is a high potential for anaerobic aquifers and the formation of sulfides by bacteria of non-human or non-animal origin. In many rural areas small-scale industry, animal husbandry and human dwellings are all contiguous, which offers the potential for sulfide formation from sediment-derived degradation of organic wastes. From these sources, only some of which were fecal sources. They concluded that assay was not suitable for control of surface water and dug well water due to the frequent presence of non-fecal coliforms. However, it was very suitable for routine control of high quality water systems, like treated community water systems or deep-tube well water, where complete absence of coliforms was required.

Desmarchelier et al. (1992) compared the H₂S water screening test and the membrane filtration fecal coliform count with Escherichia coli counts for water samples collected from household water sources and domestic drinking water in rural Malaysia. The study was undertaken to analyze 151 wells, 44 taps supplying water from the treated municipal supply and 192 domestic stored water supplies. E. coli were detected in 20% of the samples (42% of wells, 7% of tap water and 6% of drinking water). Excellent correlation (Spearman’s rank correlation rs = 0.93) was found between the fecal coliform tests for all sample types.

In this context, Castillo et al. (1994) tested that for 622 water samples by the H₂S and coliform test. They reported that 168 samples were positive by both tests (H₂S and coliform) and 179 samples were negative by both tests (H₂S and coliform). The H₂S test produced about 10% more positive samples than the coliform test but included samples that were positive for Clostridium spp. The H₂S test gave similar results at both 32°C and 35°C, indicating that temperatures in this range were not critical. Bacteria detected from H₂S positive samples included Klebsiella spp., Enterobacter spp., E. coli, Citrobacter spp., Aeromonas spp.,
Clostridium spp., Hafnia spp., Salmonella spp., Acinetobacter spp., Morganella spp. It was concluded that the simplicity and low cost of the H₂S test was applicable to tropical and subtropical potable waters.

Gawthorne et al. (1996) evaluated the H₂S test for Salmonella detection using four species grown in the laboratory and then seeded into water. They found that detection of as little as 5 CFU/100 ml was possible and longer incubation times (48 hours) increased detection of low Salmonella levels. The presence of other bacteria has no effect on Salmonella detection. The H₂S test was recommended as a presumptive test for Salmonella in drinking water in conjunction with coliform testing.

In studies of 54 conventionally treated drinking waters and corresponding raw source waters, Martins et al. (1997) found 100% agreement between total coliform and H₂S results for raw waters and 81% agreement for treated waters. In treated waters more samples were positive by the H₂S test (9 samples) than by the coliform test (7 samples), which was attributed to the presence of Clostridium perfringens in the H₂S-positive but TC-negative samples. In treated waters the H₂S and TC results were significantly positively correlated (P < 0.0001) Spearman rank correlation test but in raw waters they were significantly negatively correlated (P = 0.0008). The authors concluded that the H₂S test was a suitable indicator of potable water quality, which provided greater protection than the total coliform test.

Popp and Morris (1995); Yasushi (1998) reported that the H₂S test and its susceptibility to detect organisms of non-fecal origin was microbially induced. Microbially induced metal corrosion caused by a number of naturally occurring bacteria and fungi in microbial communities that include sulfate reducing bacteria, acid producing bacteria and other types of bacteria involved in the corrosion process.

Genthe and Franck (1999) evaluated the H₂S test for specificity, sensitivity and interference by non-target bacteria using seeded positive and negative samples. The study reported favorable results for 413 water samples from various
sources, including ground and surface water. The H$_2$S test showed 82% and 86% agreement with fecal coliform results when applied to higher quality waters with test incubation temperatures of 35 and 22°C respectively. Authors concluded that the H$_2$S test was sensitive and correlated with traditional indicator bacteria, especially fecal coliforms.

Nagaraju and Sastri (1999) tested ground water from wells of Mysore city, India for H$_2$S bacteria using the methods of Manja et al. (1982) and 24-hour incubation at 37°C. Out of 51, 37 ground water samples were positive. From these H$_2$S-positive samples the 63 bacteria were isolated, which include *Proteus mirabilis* (19), *Proteus vulgaris* (14), *Citrobacter freundii* (13), *Salmonella* spp. (8), *Klebsiella pneumoniae* (5) and *Klebsiella ozaenae* (4) were isolated.

Pillai et al. (1999) analyzed the reliability of the H$_2$S method for detecting faecal contamination in drinking water. The minimum level of faecal coliforms that could be detected and the incubation period required at various levels of contamination was studied. The range of temperatures at which the method was effective and the incubation period required at various temperatures was also determined. The H$_2$S method was found to be able to detect contamination down to a level of 1 CFU/100 ml of coliform bacteria. Although the H$_2$S method could be used at a temperature range of 20-44°C, the temperatures between 28-37°C gave faster results. An incubation period of only 24 hours was required at 37°C, which was found to be the most suitable incubation temperature. The incubation periods increased with a decrease or increase in temperature and concluded that faecal contamination in drinking water was the main cause of the waterborne disease outbreaks. Various modifications of H$_2$S tests evaluated for detection of fecal contamination using 100-ml volumes of feces diluted in distilled water to contain different levels of fecal coliform bacteria. It was concluded that the presence of cystine in the medium and higher incubation temperatures (28-44°C vs. 22°C) improved detection, with lower levels of fecal contamination (fewer fecal coliforms) detected faster.
Anwar *et al.* (1999) assessed the bacteriological quality of drinking water in Punjab and evaluated usefulness of H$_2$S strip test in comparison with multiple tube tests. Bacterial contamination was significantly higher ($p < 0.001$) in rural areas as compared to urban areas. Comparison of results for testing water samples by H$_2$S strip test and multiple tube tests revealed that H$_2$S strip was 87.24% sensitive and 100% specific for detection of bacterial contamination with a positive predictive value of 100%. It was also observed that 100% water samples negative for total coliforms were also negative by H$_2$S strip method. Moreover, with increase in number of total coliforms in the water samples, positivity by H$_2$S strip method also increased (samples with more than 10 total coliforms/100 ml were 100% positive by H$_2$S strip method) and it was suggested that, H$_2$S strip test could be used as an alternative to multiple tube test for detection of bacterial contamination of water supplies. For this purpose use of H$_2$S strip test was advocated at household level.

Jothikumar and Rao (2000) conducted a study in which simple, sensitive, rapid, inexpensive paper strip impregnated with *Salmonella* - *E. coli* medium (SEM) was formulated and placed in a test tube. When 10 ml water sample was added to the test tube it detected the fecal contamination of water samples within 16-48 h and incubated at room temperature ranges from 20 to 35°C. The positive results were indicated when the medium turned black (hydrogen sulfide production) for the presence of *Salmonella* spp. The formation of a red ring (free indole from tryptophan) after addition of few drops of Kovac's reagent showed the presence of coliform bacteria (*E. coli*). More than 600 water samples were tested with the new test (SEM) and results showed 99% agreement with that of the standard most probable number (MPN) coliform test and also proved highly successful in the field.

Moreover Manja *et al.* (2001) compared the H$_2$S test (with cystiene in the medium, different sample volumes, different incubation times and incubation at different temperatures) to MPN tests for detecting fecal contamination in 686 water samples in India. The H$_2$S test gave results comparable to the MPN test (not significantly different), with concordance in 620 (90%) samples, negative H$_2$S
test and positive MPN test (false negative) in 34 samples (4.9%) and positive H₂S test and negative MPN test (false positive) in 32 samples (4.7%). However, 21 of 23 "false positive" (negative coliform MPN) samples had coliforms in H₂S bottles. Agreement of H₂S-positive and coliform-positive samples increased from 91% at 48 hours to 95% at 72 hours. The H₂S test results were comparable for sample volumes of 20, 55 and 100 ml. Positive H₂S results were generally obtained in 18-48 hours of incubation at 25-44°C. Use of an incubation temperature below 25°C was not recommended. In these studies the following media were compared for the H₂S test: (1) original H₂S medium, (2) original medium with 250mg L-cystiene, (3) original medium with decreased peptone at 15g and added yeast extract at 2g, and (4) medium 3 with 250 mg L-cystiene and the lower peptone concentration of 15g. Based on detecting low levels of *Citrobacter freundii* and *Salmonella typhimurium* strains seeded into sterile distilled water at about 5 CFU per sample, medium formulation 3 (original medium plus 250 mg/L cystiene) was judged to give the best results based on the number of positive samples obtained.

In addition, the commercial powder form of the medium gave better results than the strip medium (liquid medium applied to paper and dried in the laboratory). It was concluded that as few as one *Salmonella* was detectable in 20 ml of sample, it was recommended for use by community workers to monitor water supply sources. At the present time there remained considerable obstacles to the widespread use of the H₂S tests because of the lack of uniformity and lack of availability in a ready-to-use form. Greater efforts to determine the optimum properties for and test conditions of H₂S tests were recommended. The other recommendations were the efforts to evaluate their validity, reliability and predictability as fecal indicators before widespread production, dissemination. The use of either commercial or made-from-scratch H₂S tests also suggested.

The sensitivity of the H₂S test referred to the minimum number of coliform forming units (CFU) required to produce a positive result per 100 ml of sample. Manja et al. (1982) tested the 20 ml samples and found that it takes 8 to 9 TC CFU per 100 ml to produce a positive result. On the other hand,
Pillai et al. (1999) determined a sensitivity level as low as 1 TC CFU per 100 ml of coliform bacteria. Grant and Ziel (1996) tested 100 ml samples and estimated a sensitivity of about 5 TC CFU per 100 ml. More specifically, they found that in every 100 ml sample, as little as 1 Salmonella typhimurium, 2 Citrobacter freundii, 2 Proteus vulgaris, produced a positive result with the H₂S test within 40 hours. Therefore, it was suggested that only one or two cells of H₂S-producing bacteria required producing a positive reaction with the H₂S test.

The H₂S method has been tested by Nair et al. (2001) for treated drinking water and was found to have a good correlation with the standard methods. The study was aimed to assess the suitability of H₂S method for testing different sources of drinking water. The experiment analyzed 121 rainwater samples, 17 borewater samples, 41 catchment water samples and 74 remote aboriginal community water samples. Results were compared with the results using standard procedures for testing total coliforms, Escherichia coli and Salmonella spp. Rainwater, borewater and catchment water samples gave true results of 78.5%, 82.3% and 80.5% respectively while the treated and untreated community samples gave true results of 93.7% and 84.6% respectively. It was concluded that in the developing countries where the acceptable level of total coliform is <10 MPN, the H₂S method would be a good test to identify microbial contamination. In other regions, the H₂S method could be used as a screening test for drinking water supplies.

Rijal and his colleagues (2000) developed and evaluated two modifications of the H₂S test, a MPN version using replicate sample volumes of 1, 10 and 100 ml and an enumerative version for colonies on membrane filters by using an agar medium. When both H₂S tests were compared to each other and to coliforms and E. coli in rainwater cisterns of drinking water, both H₂S methods gave results comparable to E. coli. They compared two versions of the H₂S test, a paper strip MPN and a membrane filter enumeration on agar medium, to each other and to the occurrence of total coliforms and E. coli in samples of cistern rainwater, ground water and stream water. Similar detection of bacterial contamination was achieved

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by the MPN, MF version of the H$_2$S test and E. coli, although total coliforms were detected in more samples than were either E. coli or H$_2$S bacteria.

Tewari et al. (2003) evaluated simple microbial test comprising H$_2$S paper strip test, presence-absence (PA) test and fluorogenic bile broth (BB) test. It was performed directly at 44.5°C and compared with MPN method for detection of fecal coliforms in 173 drinking water sources. BB and PA tests were comparable with standard MPN method, whereas, poor compliance was noted for H$_2$S test. After comparing PA test with standard MPN test, only 15% disagreement was detected, whereas, highest disagreement of 40% was observed in case of H$_2$S test. BB test was found to be highly sensitive as only 7.8% disagreement with that of standard MPN test was found. Three hundred cultures obtained from positive tests were identified in order to evaluate the specificities of test used in detection of fecal indicator E. coli. BB test was also found highly specific in detection of indicator organism as compared to PA and H$_2$S test. Among the organisms isolated from BB test, 84.4% of them were identified as E. coli when compared to 43.4 and 33.3 in PA and H$_2$S test, respectively. The low incidence of recovery of E. coli (18.1%) for the standard MPN method placed doubt on the validity of its application in tropical areas. The result of this investigation suggested that BB performed directly at 44.5°C could be suitable and cost effective to assess the microbiological quality of drinking water in India and other tropical countries.

Anwar et al. (2004) assessed the bacteriological quality and chlorination status of drinking water in Lahore by using H$_2$S test. A total of 2160 water samples from distribution system were tested from nine different localities of Lahore. These localities represented areas with different socioeconomic conditions (SEC). Twenty water samples were tested from each locality from the same taps each month. All the water samples were subjected to H$_2$S strip test for determination of bacteriological contamination as well as orthotolidine test for detection of chlorine. Four hundred and forty-six (20.64%) samples were positive for bacterial contamination. It was observed that contamination was the maximum in low SEC areas (32.22%), followed by intermediate SEC areas (18.47%) and high SEC
areas (11.25%). The difference was found to be statistically significant (p<0.01) among different areas. Only 27.73% samples were chlorinated. Positivity of samples for chlorine was the lowest in areas with low SEC (20.69%) and highest (32.77%) in areas with high SEC, the difference being statistically significant. Maximum contamination was present in samples tested during summer months (June-August) of the year (31.11%), followed by autumn months (September-November) of the year (20.9%), spring months (March-May) of the year (18.7%) and winter months (December-February) of the year (11.85%). Samples tested during summer months showed the highest positivity (77.96%) for chlorine (p<0.001) as compared with other months of the year. Among chlorinated samples 12.32% showed bacterial contamination. However, contamination was significantly higher (p<0.001) among non-chlorinated samples.

For evaluating the efficiency of H₂S test with 90 water samples, Pathak and Gopal (2005) collected water from 40 pipes supplies, 20 open wells, 15 hand pumps and different surface water bodies (river, streams and ponds). Sterilized modified culture medium in glass vials was inoculated with 100 ml of each sample and incubated at 20, 25, 30, 35 and 44 °C for 18, 24, 42, 48, 66 and 72 h. and compared with MPN and fecal coliform per 100 ml. H₂S positive result was exhibited by 78% of samples. Coliform (>10) and fecal coliform/100mL were also detected in 59% of samples. Maximum H₂S positive results (100%) were found with well and surface water samples incubated at 30, 35 and 44 °C for 18 h. Coliform (>10) and fecal coliform/100 ml were also detected in most of these samples. Pipes supplies (60%) and hand pump (73%) also exhibited considerable H₂S positive results. Thus it was concluded that the modified H₂S test may proved a useful alternative indicator of fecal contamination for water quality surveillance and screening of large number of water samples in short duration.

Roser et al. (2005) compared the H₂S test’s ability to detect and quantify fecal contamination in an aquifer impacted by septic tank leachfields with measurements obtained concurrently using conventional bacterial indicators, coliphages, fecal sterol biomarkers, Cryptosporidium and Giardia. H₂S testing
detected a contamination gradient ranging from high (septic liquid) to moderate (exfiltration zones), to background (e.g. domestic bores), corresponding to indicator removal + dilution by factors > 10. Presence/absence tests could not distinguish between heavily and slightly contaminated waters, whereas multi-tube testing (e.g. 10 x 10 ml arrays) did. It was concluded that while the WHO review concerns were justified, the 'H₂S test' was being advanced for microbiological water quality testing where conventional coliform-based methods were impractical or too expensive. They concluded that the H₂S test performance showed relevance in sanitary survey work, could be improved by employing an MPN approach and had potential to aid in the protection of source water and identifying contaminated groundwater.

Hirulkar and Tambekar (2004a and b); Tambekar and Hirulkar (2006) assessed the rapid H₂S test with MPN technique for bacteriological analysis of drinking water. A good correlation was recorded between H₂S test, MPN count and Eijekman's test. Total 510 drinking water samples were analyzed by standard MPN technique, Manja's modified H₂S test and Eijekman's test. All the water samples that had MPN count zero were negative for H₂S test. Out of 510 samples analyzed, while 269 were positive for MPN test (>10 coliforms /100 ml), 234 were positive for H₂S test and 220 water samples were confirmed TTC by Eijekman's test. However 234 water samples were positive and 238 were negative by both H₂S and MPN test (<10 coliforms /100 ml) indicating 92.5% agreement between the results in both methods. The positive test percentage of agreement between H₂S test and MPN method of coliforms was 87% and 94% with Eijekman's test. Thus H₂S test was found to be 92.5% efficient with a standard MPN test and proved to be an alternative method for assessing the water quality of potable water (Hirulkar and Tambekar 2006b).

According to Hirulkar and Tambekar (2005a and b); Tambekar et al. (2006g), Bacterial contamination of drinking water is significant problem in hotels and restaurants and unhygienic practices may leads to bacterial contamination and water become nonpotable for drinking purposes. Washing of storage
container, hygienic condition of hotels and restaurants' owners and workers, uniform of worker, number of customer per day, health education of workers and owners also affect the potability of drinking water available in hotel and restaurants. They analyzed 225 drinking water samples from hotels and restaurants in Amravati city for determination of bacteriological quality. Out of these, 143 (63.5%) had MPN count more than 9 (i.e. nonpotable) and 82 (36.4%) had MPN were confirmed faecal contamination by Eijkman's test.

A study on suitability of H₂S field test to detect fecal contamination in drinking water was also performed by Hirulkar and Tambekar (2005c and 2006a). They analyzed 635 water samples from various sources at room temperature and at 37°C after 18 h, 24 h, and 48 h of incubation. The H₂S test showed 216%, 85%, 96% and 85% correlation with Eijkman's test, membrane filter technique, most probable number (MPN) test for coliform and membrane filter technique (MFT), respectively (Tambekar et al., 2006f).

Tambekar et al. (2006b) analyzed 1000 water samples from various sources such as tube wells (340), open wells (240) and hotels and restaurants (320) for the coliform water contamination. The study revealed that the 96 strains of Salmonella typhi were isolated and tested against selected antibiotics. Most of the Salmonella species were sensitive to norfloxacin, ciprofloxacin, levofloxacin, kanamycin, streptomycin, ceftazidime and chloramphenicol and resistant to linezolid, gatifloxacin, amoxiclav, cefepime, ampicillin, nalidixic acid and co-trimoxazole. It was recorded by Hirulkar and Tambekar (2006c); Tambekar et al. (2006c) that 425 water samples were contaminated with total coliform by MPN technique. Out of them 85 strains of thermotolerant Escherichia coli 51 (60%) from open well, 23 (32%) from tube well and 11 (13%) from hotels and restaurants were isolated and identified. These isolates showed maximum resistance to ofloxacin followed by novobiocin, cefdinir and ciprofloxacin. The azithromycin, gentamycin, amikacin, chloramphenicol, co-trimoxazole and tetracycline were the most effective while the ofloxacin, novobiocin, cefdinir and ciprofloxacin were the least effective against E. coli isolates.
(Tambekar et al., 2005). A total of 63 strains of *Proteus* species, 35 *Proteus mirabilis* and 28 *Proteus vulgaris*, were isolated and identified from these contaminated drinking water. Comparatively higher degree of resistance was recorded in *Proteus* species isolated from open well followed by tube wells and hotels and restaurants (Tambekar et al., 2006d). The higher level of resistance to these antibiotics and antibiotic resistant bacterial emergence in drinking water source due to improper and higher use of antibiotics was recorded. The 210 water samples were analyzed and 40 strains of *Pseudomonas aeruginosa* were isolated and identified from various drinking water sources from different localities in Amravati city. Antimicrobial susceptibility tests were carried with 24 antibiotics, which showed 47% resistance and 53% sensitive (Tambekar et al., 2006a).

During bacteriological quality assessment of ground water in Amravati by Tambekar et al. (2006e), 710 water samples were analyzed, 355 each from open wells (OWs) and tube wells (TWs) of the different localities in Amravati city. Out of the total TW water samples, 81% were contaminated by MPN test, 74% by MFT and 10% contained thermotolerant *E. coli*. Out of 355 OW water samples, 82% were confirmed contaminated by MPN test, 69% by MFT and 13% contained thermotolerant *E. coli*. Analysis indicated that the maximum bacterial contamination was recorded during monsoon as compared to pre-monsoon and post monsoon in OW. A total of 340 drinking water samples from hotels and restaurants also analyzed for bacterial contamination, out of them, 69.1% were non-potable by MPN method, 73.2% by MFT indicated presence of *E. coli* and 18.2% showed presence of thermotolerant *E. coli* of human fecal origin. Study concluded that poor hygiene behaviors such as improper method of storage, handling and serving deteriorated the quality of drinking water which can be improved by imparting water hygiene behavior education to hotel and restaurant owners.

In most comparative studies there were always samples that yield positive results for other microbiological tests and negative H₂S tests and vice versa. However, such results are not unexpected. For one, the various tests measure
different things and do not always employ the same sample volumes. Furthermore, when the levels of microbial contamination are low, it is statistically possible for one sample volume to contain bacteria of interest and for another to not contain them. Where study data were subjected to statistical analysis, most studies found high associations (e.g., correlation) between fecal indicator bacteria (e.g., *E. coli*) and positive H$_2$S results. Giving the previously discussed ability of a large variety of heterotrophic bacteria to produce a positive H$_2$S test, the observed correlations suggested that in most natural and treated waters the majority of the H$_2$S producers come from organisms associated with the human or animal digestive tract. A false positive is less likely to lead to a risk of disease because it would result in the tested water either not being used or subject to additional testing.

The various forms of the H$_2$S test differ in the medium and its preparation procedures, media format (dried onto paper strip, use as powder and agar medium), test format (presence-absence, MPN and membrane filter), sample volumes, incubation times and incubation temperatures. Though various people test the validity of the H$_2$S test with MPN or MFT, further validation is required to make the test as a standard test for detection of quality of drinking water. It needed to standardize this method for use in developing countries. The results of several studies indicated that various forms of H$_2$S test have been evaluated and are being used. Hence attempt was made to evaluate the H$_2$S test with standard water analysis test.