

## Antimicrobial Activity of Leaves of Medicinal Plants on Bacteria Present in Drinking Water



### Chemistry

**KEYWORDS :** Water, Pollution, Bacteria, Medicinal Plants.

**Dr. A. Vasundhara**

Reader in Chemistry & S.K.R.College for women, Rajahmundry, A.P, INDIA.

**N. Aruna Kumari**

Assoc. Prof., Dept of HBS, GIET, Rajahmundry, A.P, INDIA.

### ABSTRACT

*An essential component of managing water resources and the environment is that of monitoring and analysis of samples taken from the environment. Without the chemical and microbiological analysis of water samples, it would not be possible to determine the status and safety of drinking water supplies, which are so essential requirement for the well-being of communities, and the sustenance of life. Water resources are subject to ever-increasing pollution pressures, paralleling an overgrowing demand for water for drinking purposes and other uses. The determination of the chemical and microbiological quality of the water supplies is essential to establish the need and adequacy of treatment, and to safeguard human health. Water having the impurities can be treated with some chemicals to get pure water. From the literature, it is clear that not only chemicals, other plant products also can be used to get pure water. Basing on the information available, the author tried to inhibit the growth of four bacteria using tender leaves of plants and their extracts in acetone and chloroform. In this present work, it is given the particulars of the analysis of river Godavari water and inhibition of growth of microorganisms using tender leaves of different plants.*

### INTRODUCTION

Every one of us know how important and precious the water is. No life can exist without water, since water is as essential for life as air. It has been estimated that two-third of human body is constituted of water. Water is absolutely essential not only for survival of human beings, but also for animals, plants and all other living beings. Further, it is necessary that the water required for their needs must be good, and should not contain unwanted impurities or harmful chemicals or bacteria in it. So as to keep the diseases away and thereby promoting better health, it is essential to make water clean and pollution free. From the literature, it is clear that most of the plant parts are having medicinal values to cure diseases. So, making use of those properties, the author has tried to inhibit the growth of certain microbes by using tender leaves of some plants mentioned below and by taking dry leaf powder extracts in acetone and chloroform.

### EXPERIMENTAL

In this present work the author has attempted to see the effect of plants on the growth of microorganisms present in river water. The four microorganisms are Klebsiella, Vibrio, E-coli and Staphylococcus. The leaves collected from the following trees.

1. Citrus auratifolia- Lemon
2. Aloe vera- Kalabanda
3. Cantharthus roseus- Madagascar periwinkle (Billaganneru)
4. Aegle marmelos- Bengal quince, Stone apple (Maredu)
5. Croton Tigulum- Purging Croton (Nepalam)
6. Psidium guajava- Guava
7. Mangifera Indica- Mango
8. Ocium americanum- Holy basil or Tulasi
9. Syzygium Cumini- Black plum (Neredu)
10. Piper betel- Betel

### IDENTIFICATION OF MICROORGANISMS

Identification was based on morphological and biochemical based characterization.

Morphological identification: Colony characters, grams staining, spore staining and mobility test.

### MATERIALS REQUIRED

- o Glass slides
- o Vaseline
- o Cover slips
- o Microscopes
- o Immersion oil (Ceder wood oil)
- o Crystal violet
- o Gram's iodine
- o 70% ethanol
- o Malachite green
- o Glucose peptone broth, Nutrient agar, SS agar and McCo-

nkey agar

- o Tryptone water, TCBS agar, Endo agar, Cetrimide agar
- o Simmon citrate agar, Skimmed milk agar, Mannitol salt agar
- o Methylene red indicator
- o Saffranine
- o P-di methylene amino Benzaldehyde
- o Hydrogen peroxide
- o Tetra-methyl-p-phenylenediamine dihydrochloride
- o Urea broth
- o Phenol red
- o Glucose, Sucrose and lactose broths
- o 40% KOH
- o 5% alcoholic  $\alpha$ -Naphthol
- o Oxidation-fermentation medium
- o Starch agar
- o Lipase agar
- o Gelatinase agar
- o 1N NaOH

### MORPHOLOGICAL CHARACTERS

#### GRAM'S STAINING:

The smear on a glass slide is covered with a few drops of one of the primary stains. Gentian violet is a mixture of methyl violet and crystal violet. The primary stain renders all the bacteria uniformly violet. After a minute of exposure to the staining solution, the slide is washed in water. The smear is treated with a few drops of Gram's iodine and allowed to act for a minute. This results in the formation of a dye-iodine complex in the cytoplasm. Gram's iodine serves as mordant. The slide is again washed with water and then decolorized in absolute alcohol. Acetone is a potent decolorizer and when used alone can decolorize the smear in 2-3 seconds.

A mixture of ethanol and acetone acts more slowly than pure acetone. Decolorization is the most crucial part of Gram staining and errors can occur here. Prolonged decolorization can lead to over decolorized smear and a very short decolorization period may lead to under-decolorized smear. After the smear is decolorized, it is washed in water without any delay. The smear is finally treated with few drops of counter stain such as dilute carbon fusion, neutral red or safranin.

#### MOTILITY CONFIRMATION BY HANGING DROP METHOD:

Using a tooth pick, Vaseline is applied on the edges of clean cover slip. It is kept on the objective lens of the microscope. A loop full of the culture to be tested is placed in the center of the prepared cover slip. The clean concavity slide is turned upside down over the drop on the cover slip so that the Vaseline seals the cover slip to the slide around the concavity. The preparation is kept in the microscope slide holder and aligned it using the naked eye.

**BIOCHEMICAL BASED IDENTIFICATION:****METHYL RED TEST:**

Some bacteria perform mixed acid fermentation. The by-products are mixtures of large amounts of stable acids. Other fermentative organisms produce smaller amounts of less stable acids. The Methyl-red test is used to perform mixed acid fermentation.

MR-VP broth contains glucose, peptone and a phosphate buffer. Organism that perform mixed acid fermentation produce enough acid to overcome the buffering capacity of the broth, so a decrease in pH results. Organisms that perform other kinds of fermentation cannot overcome the buffering capacity of the broth.

After incubation, the pH indicator Methyl Red is added to the broth. The methyl red is red at pH below 4.4 (this would be a positive result) and yellow at pH above 6.0. An orange color indicates an intermediate pH and would be considered a negative result.

**PROCEDURE:**

Two MR-VP broths are taken. One broth is inoculated using aseptic technique. The other broth is left un-inoculated. It is incubated for two to five days. Two broths are taken outside. A dropper full of Methyl red is added to each broth. The color of the broths are observed.

**VOGES-PROSKAUER'S TEST:**

Some microorganisms especially Enterobacteriaceae members can be able to produce 2,3-butanediol from the source like monosaccharides.

The mechanism of chemical reaction is as follows:

Glucose → pyruvate → acetolactate → acetoin → 2,3-butanediol and acetyl methyl carbinol. This carbinol was identified by 40% KOH and 5% alcoholic  $\alpha$ -Naphthol by formation of pink colored ring at the surface of the medium.

**PROCEDURE:**

VP broth is taken from the incubator. One broth is inoculated using aseptic technique. The other broth is left without inoculation. Incubated at appropriate temperature where the organism grows for 2 to 5 days. 1.0ml of 40% KOH and 1.0ml of 5% alcoholic  $\alpha$ -Naphthol to each broth are added and results were interpreted by formation of pink colored ring at the top of the solution.

**INDOLE TEST:**

This test is done to determine if bacteria can breakdown the amino acid tryptophan into indole.

This test is done to determine if bacteria can breakdown the amino acid tryptophan into indole. SIM media or TSB is inoculated using a transfer needle. After incubating the bacteria for at least 48 hours, Kovac's reagent is added to the media to detect if indole has been made by the bacteria. The development of a red/pink layer on top of the media is a positive result (the bacteria can breakdown tryptophan to form indole), failure to see a red layer is a negative result (indole was not formed from tryptophan).

**PROCEDURE:**

Tryptophan broth was prepared. Sterilized by autoclaving at 121°C for 15 minutes. A loop full of inoculation medium was added to the sterilized medium and incubated for 24 hours. 1.0ml of Kovac's reagent was added (p-dimethyl aminobenzaldehyde). Cherry red colour ring appearance is positive test.

**CITRATE TEST:**

The slant is inoculated and bacteria that are able to utilize citrate as a fuel will catabolise the citrate in the medium and release an end product that is basic (alkaline). The indicator Bromothymol blue is blue shown above pH 7.6 and green at pH values below 7.6. If citrates are utilized, the medium pH will rise

and the medium will turn from green to blue.

**PROCEDURE:**

Simmons citrate agar slant is used for this test. The slant is prepared such that citrate is the only carbon source, thus forcing the organism to use it as a nutrient. The medium also contains a pH indicator called Bromothymol blue.

The organism was streaked on the surface of citrate medium and it was allowed to incubate at 37°C for 24 hours. The change of color of the medium from green to blue was observed.

**OXIDATION - FERMENTATION TEST:**

The purpose of this test is to determine whether an organism attacks sugars (in this case glucose is used) by fermentation or oxidation. Two tubes of Hugh & Leifson's medium are used. In one tube the medium is covered with Vaseline. If the organism is an oxidizer, it will produce acid only in the open tube (without Vaseline). If it is a fermenter, it will produce acid in the Vaseline covered tube and in the open tube. Some aerobic bacteria may use the peptone in the medium, producing ammonia, with the result of alkalinity (blue) in the top part of the open tube. The indicator used is bromothymol blue.

**SUGAR FERMENTATION TEST:**

4 types of sugars were used for fermentative metabolic activity. Glucose, sucrose, lactose and mannitol used for this test. Peptone carbohydrate solution was used. A drop of inoculum was added to the autoclaved media and incubated 24-48 hours to detect the acid production by changing the medium color from red to yellow because of the indicator phenol red. Sometimes some bacteria are able to produce gas also, that gas production can be identified by placing a Durham's tube in reverse condition. The produced gas will be gathered into the inverted tube.

**ENZYME ACTIVITY TEST:****AMYLASE TEST:**

This test is used to detect the enzyme amylase, which breaks down into starch. After incubation, the plate is treated with Gram's iodine. If starch has been hydrolyzed then there is a reddish color or a clear zone around the bacterial growth. If it has not been hydrolyzed then there is a black/blue area indicating the presence of starch. Inoculating loop is used to spread bacteria onto plate surface. After the bacteria have grown, few drops of Gram's iodine is added to the plate and looked for the color.

**PROCEDURE:**

Starch nutrient agar was prepared and sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the sterilized media is taken in the Petri plates and streaked the organism in the centre of the medium for 24-72 hours. After incubation the indicator 5% iodine solution is added on to the plate. Clear hallow colored zone against dark violet colored background indicates the positive result.

**LIPASE TEST:**

Nutrient agar was prepared and the medium was supplemented with 2% tributyrin and allowed to sterilize the medium by autoclaving. After sterilization the test organism was streaked on the centre of the plate and allowed to incubation for 24-48 hours. Clear zonation was observed around the oily background, indicates positive test.

**UREASE TEST:**

This test is used to detect the enzyme urease, which breaks down urea into ammonia. Ammonia is a base and thus will raise the pH of the medium if it is present. This change in pH is indicated by a pH indicator called phenol red which is present in the medium. A colour change from yellow to bright pinkish red is positive; lack of colour change is a negative result. The liquid medium is inoculated with a transfer loop.

**PROCEDURE:**

2% peptone water was prepared and a pinch of potassium hydrogen phosphate salt is added and sterilized. After cooling the medium, 10% membrane filtered urea solution is added and the

medium was dispensed into the tubes and the test organism was inoculated and incubated for 24-48 hours. After incubation phenolphthalein indicator was added. The colorless indicator turns to red color because of formation of ammonia from urea by a novel enzyme urease which turns the pH neutral to alkaline indicates the positive test.

**GELATINE TEST:**

This medium is used to test if bacteria can digest the protein gelatin. To digest gelatin, the bacteria must make an enzyme called Gelatinase. To inoculate this medium, a transfer needle is used to stab the gelatin. After incubating the inoculated media for at least 48 hours, the tube is transferred into a refrigerator. The tube is completely chilled before observation. If the medium is solid after refrigeration, then the test is negative. (the bacteria did not digest gelatin) if the medium is liquefied even after refrigeration, then the test result is positive. The bacteria are able to digest gelatin.

**PROCEDURE**

Gelatinase agar was prepared and autoclaved, freshly poured into the Petri plates.

A loop full of inoculum was used as a single streak and incubated the plates for 24-48 hours. After that 15% HgCl<sub>2</sub> dissolved in 20% HCl is added to produce clear zones.

**ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS:**

12 different medicinal plants have taken for the above activity. The botanical names of plants along with their local or regional names are given above.

Different pathogens have been isolated from waters ample from the sacred river Godavari. The pathogens are E.coli, Staphylo-

coccus aureus , Klebsiella and Vibrio. These can be isolated by using selective media.

- E.Coli- ENDO Agar medium
- Staphylococcus aureus - Salt nutrient agar medium
- Vibrio species- TCBS agar medium
- Klebsiella species- EMB –eosine methylene Blue agar medium

**TRANSPARENT MEDIUM:**

Venkatraman-Ramakrishnan (VR) medium, Carry –Blair medium and Autoclaved sea water.

**ISOLATION AND IDENTIFICATION OF PATHOGENIC MICRO-ORGANISMS BY GROWING THEM ON SELECTIVE MEDIUM:**

1.0ml of River Godavari water sample was collected and diluted by serial dilution method. The sample was serially diluted up to 5dilutions. 1.0ml of sample was taken from the dilution tubes and growing them on selective media. Four types of selective media were used to isolate pathogenic strains. Endo agar, Eosin methylene blue agar for isolation of E-coli, salt nutrient agar and Mannitol salt agar for isolation of staphylococci, TCBS agar for isolation of vibrio and Eosin methylene blue agar for klebsiella.

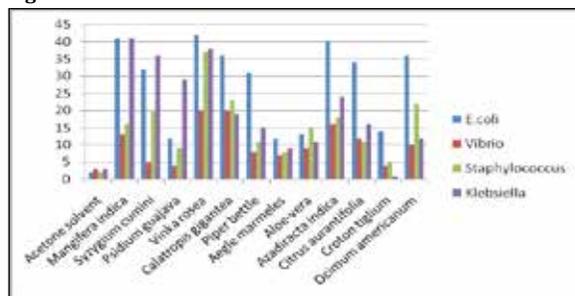
**PREPARATION OF EXTRACTS.**

The young and healthy leaves of selected medicinal plants were cut into small pieces and shade dried at room temperature for 15 days to remove moisture content. They are finely powdered. Finely powdered plant materials were successively extracted with organic solvent acetone and chloroform. Acetone based on order of polarity using Soxhlet apparatus. 2 types of organic solvents were used for extraction namely acetone and chloroform. The extracts were obtained and subsequently concentrated under reduced pressure to get their corresponding residues based on the intensity of the extracts solvents can be added to dilute.

**TABLE-1:Anti microbial activity (in mm) with acetone extract for water taken at Kotilingala ghat**

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	2	41	32	12	42	36	31	12	13	40	34	14	36
Vibrio	3	13	5	4	20	20	8	7	9	16	12	4	10
Staphylo coccus	2	16	20	9	37	23	11	8	15	18	11	5	22
Klebsiella	3	41	36	29	38	19	15	9	11	24	16	1	12

**Fig-1**



**TABLE-2: Anti microbial activity (in mm) with chloroform extract for water taken at kotilingala ghat**

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	3	25	12	8	30	24	6	7	4	25	13	6	10
Vibrio	1	11	6	5	15	8	2	4	3	15	6	2	4
Staphylo coccus	2	16	16	14	19	17	8	10	4	25	9	8	6
Klebsiella	1	14	9	7	14	7	3	8	2	17	10	11	8

Fig-2

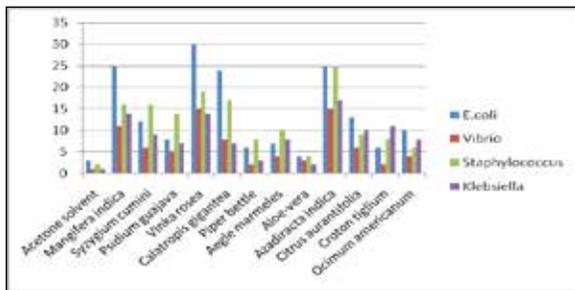


TABLE-3: Anti microbial activity (in mm) with acetone extract for water taken at Puskaraghat

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinaka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	2	35	35	12	44	41	30	12	15	40	34	14	36
Vibrio	3	13	6	2	19	17	9	7	9	15	12	4	10
Staphylo coccus	2	14	18	8	38	23	15	6	13	21	11	5	22
Klebsiella	3	42	36	28	40	19	14	6	15	23	16	1	12

Fig-3

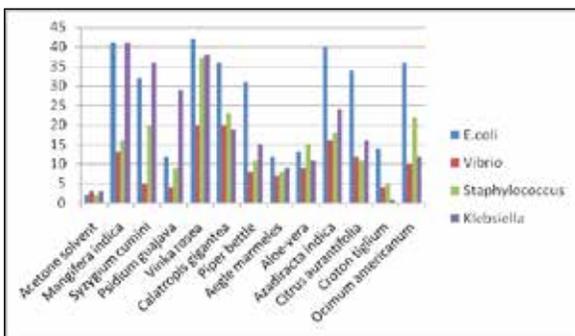


TABLE-4: Anti microbial activity (in mm) with chloroform extract for water taken at Puskaraghat

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinaka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	3	23	13	8	26	22	6	7	4	24	13	6	10
Vibrio	1	10	7	5	13	6	2	4	2	16	6	2	4
Staphylo coccus	2	15	13	11	15	13	8	11	4	23	9	8	6
Klebsiella	1	11	9	7	13	6	4	6	3	18	10	11	8

Fig-4

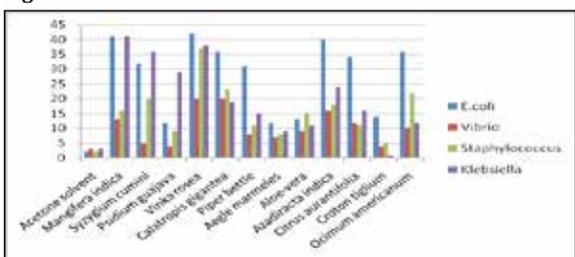


TABLE-5: Anti microbial activity (in mm) with acetone extract for water taken at Gouthami Ghat

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	2	37	32	11	40	35	29	12	13	39	34	14	36
Vibrio	3	13	5	1	19	17	9	7	9	16	12	4	10
Staphylococcus	2	16	16	8	36	23	11	6	12	19	11	5	22
Klebsiella	3	42	40	28	38	19	15	8	14	23	16	1	12

Fig-5

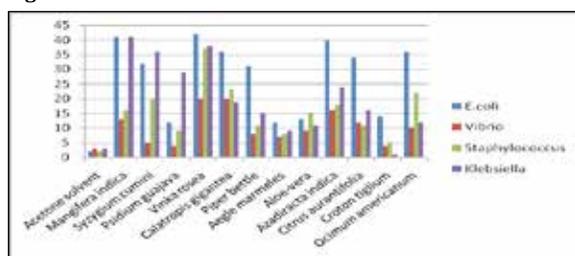
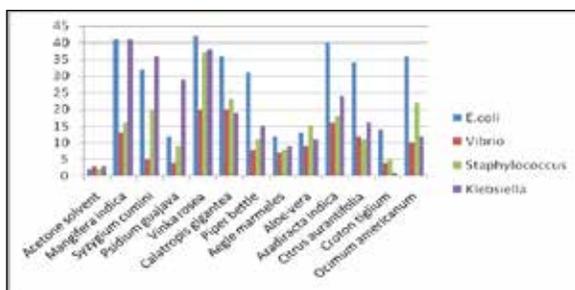


TABLE-6: Anti microbial activity (in mm) with chloroform extract for water taken at Gouthami ghat

Name	Acetone solvent	Mangifera indica	Syzygium cumini	Psidium guajava	Vinka rosea	Calatropis gigantea	Piper bettle	Aegle marmeles	Aloe-vera	Azadiracta indica	Citrus aurantifolia	Croton tiglium	Ocimum americanum
E.coli	3	25	12	9	28	22	4	9	4	24	13	6	10
Vibrio	1	10	7	4	13	7	3	4	3	18	6	2	4
Staphylococcus	2	16	13	11	19	17	8	11	3	24	9	8	6
Klebsiella	1	14	9	7	13	7	5	5	3	16	10	11	8

Fig-5



**CONCLUSION:**

The plants Betel, Tulasi, Black plum, Lemon, Neem and Mango are effective to inhibit the growth of pathogenic microorganism E-coli. Vinka rosea is highly effective to inhibit the growth of E-coli where as the plants Bengal quince or Stone apple, Purging Croton and Guava are not effective to control the growth of E-coli.

The plants Betel and lemon are effective to inhibit the growth of Klebsiella. Mango and Vinka rosea are highly effective to inhibit the growth of Klebsiella where as the plants Tulasi, Bengal quince or Stone apple, Purging Croton, Black plum, Guava and

Neem are not effective to control the growth of Klebsiella.

Because of the growth of low content of bacteria i.e Vinka rosea where as the plants Betel , Bengal quince or Stone apple, Purging Croton and neem are not effective to control the growth of Klebsiella.

Because of the low amounts of bacteria i.e Vibrio and Staphylococcus in water sample, it is unable to identify the result on treatment with plant leaves.

The plants Tulasi, Bengal quince or Stone apple, and Guava are effective to inhibit the growth of Vibrio , Black plum is highly effective to inhibit the growth of Vibrios. By using the leaves of plants Betel, Vinka rosea, Purging Croton, Lemon, Neem and Mango no growth is found.

On 24hours of incubation, the plants Betel, Tulasi, Vinka rosea, Black plum, Lemon, Neem and Mango are very effective to inhibit the growth of 4 pathogenic microorganisms.

On 48 hours incubation the plants Tulasi, Vinka rosea, Guava, Lemon and Mango are effective to inhibit the growth of 4 pathogenic microorganisms.

By comparing these 2 results the plants Tulasi, Vinka rosea, Black plum, Lemon and Mango are highly effective

## REFERENCE

- Al-Moagel, M. A., Evans, D. G., Abdulghani, M. E., Adam, E., Evans, D. J., Malaty, H. M. and Graham, D. Y. (1990). Prevalence of *Helicobacter pylori* (formerly *Campylobacter*) infection in Saudia Arabia and comparison of those with and without upper gastrointestinal symptoms. *American Journal of Gastroenterology* 85, 944-948. | Armstrong, J. L., Shigeno, D. S., Calomiris, J. J. and Seidler, R. J. (1981). Antibiotic resistant bacteria in drinking water. *Applied and Environmental Microbiology* 42, 277-283. | Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45, 493-496. | Begue, R. E., Gonzales, J. L., Correa, G. H. and Tang, S. C. (1998). Dietary risk factors associated with the transmission of *Helicobacter pylori* in Lima, Peru. *American Journal of Tropical Medicine and Hygiene* 59, 637-640. | Boon, P. I. and Cattanaach, M. (1999). Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, South-Eastern Australia. *Letters in Applied Microbiology* 28, 164-168. | Brown, L. M. (2000). *Helicobacter pylori*: Epidemiology and routes of transmission. *Epidemiology Reviews* 22, 283-297 | |