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MICROBIAL LIMIT TEST OF DIFFERENT PATHOGENS FOR WATER SAMPLE

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ABSTRACT:-

Bacteriological water analysis is a method of analyzing water to estimate the number of bacteria present and, if needed to find out what sort of bacteria they are it represent are aspect of water quality. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inference about suitability of the water for these concentration. The pure water free from of pathogen organism is called potable water.it is used for drinking water is obtained from rivers, streams, lake, wells, borewells e.t.c. normally, the coliform bacteria are considered as the indicators of pollution of drinking water. Theses live in the human colon of these Escheria coli is important and it is released through feaces. The presence of Escheria coli on the water indicates pollution. Potability of water is determined by bacteriological test.

INTRODUCTION:-

Water is essential to life, but they people do not have access to clean and safe drinking water and many die water born bacterial infection. In this review of general characterization of the most important bacterial diseases transmitted through water cholera, typhoid fever and bacillary dysentery is presented, focusing on the biological and ecology of the causal agents and on the diseases. Characteristic and their life cycle in the environments. The importance of pathogens Escherichia coli strains and emerging pathogens in drinking water transmitted diseases is also briefly discussed. Microbiological water analysis is mainly based on the concepts of fecal indicator bacteria. The main bacteria present in human and animal feces (focusing on their behavior in their host and in the environment) and the most important fecal indicators bacteria are presented and discussed.(focusing on the advantages and limitation of their use as markers).important sources of bacteria sources of bacterial fecal pollution of environment waters are also briefly indicated in the topic it is discussed which indicators of fecal pollution should used in current drinking water Microbiological analysis. Routine basic Microbiological analysis of drinking water should be carried out by assaying the presence of Escherichia coli by culture

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method whenever financial resources are available, fecal coliforms determination should be completed with the quantification of Enterococci more studies.

METHODLOGY:-

There are two types of methods of microbial limit test –

1. Direct inoculation – in this method sample is directly inoculated into the medium and that are incubated.
2. Membrane filtration method – in this method sample solution is filtered with filtered membrane and then this filter membrane is transfer to the freshly prepared sterilized media.

Above methods are performed for.

I. Total Bacterial count.
II. Total Fungal count.
III. Pathogen testing.

Direct inoculation methods:- Test for pathogens.

1. Escherichia coli.
2. Salmonella
3. Pseudomonas aeruginosa
4. Staphylococcus aureus.

- Escherichia coli:-

1. It is facultative anaerobic, Gram negative rod shaped bacteria.
2. Bacteria can grow in the absence of oxygen.
3. Under anaerobic condition they can grow by means of fermentation.
4. The media is used is selective media is MacConkey agar,Eosin Methylene Blue ager.
5. The infection caused by these organism is urinary tract infection, intestinal diseases.

TEST:-

1. Aseptically transfer 10ml water sample to 90ml soyabean casein Digest Medium.
2. Incubate at 35-37c for 18-48 hrs.
3. After incubation, observe and carry out primary test.

PRIMARY TEST :-

1. Add 1ml enrichment culture to 10ml MacConkey broth containing inverted Durahm's tube.
2. Incubate at 40-45c for 18-24 hrs.

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3. If the content of the tube shows acid & gas formation, carry out confirmatory test. Acid production is indicated by change in colour broth from purple to yellow & gas is accumulated at the top of Duraham's tube.

SECONDRY TEST :

1. Inoculate 0.1ml from these tube to 5ml of peptone water.
2. Incubate tubes at 35-37c for 18-24 hrs.
3. INDOLE TEST : After incubation add 0.5ml of Kovac's reagent into peptones water tubes. Shake well & allow standing for 1 min. if red coloured ring is produced in reagent layer, INDOLE test is confirmed.
4. Presence of red non mucoid colonies of Gram negative rods surrounded by reddish precipited. Zone of MacConkey agar indicates of E.Coli.
5. If the colonies described above are found transfer the suspect colonies individually by streaking to the surface of eosin for Methylene blue agar on petridish. Incubate the plates at 35-37c for 18 -24hrs. if the colonies exhibit metallic shine under refiect light & blue black appeareance confirm the presence of E.Coli.

- SALMONELLA:-

1. It is Gram negative, motile, flagellated, noncapsulated, nonsporing bacilli.
2. It is animal & human pathogens.
3. The media is used is Brilliant Green Agar, Bismuth Sulphite Agar, Deoxycholate citrate Agar.
4. It causes food poising, enteric fever.

TEST:

1. Aseptically transfer 10ml of water to 90ml of soyabean casein Digest Medium.
2. Incubate at 35-37c for 18-48 hrs.

PRIMARY TEST:

1. 1ml of enrichment culture is inoculated in 10ml of tetrathionate bile brilliant green broth and 10ml fluid selenite cystine broth and then incubated at 35-37c for 18-24hrs.
2. If growth is observed, then sub culture is done from this culture on at least two or three selective agar media, Brilliant green agar(BGA), Bismuth Sulphide Agar(BSA), Deoxycholate citrate Agar(DCA).
3. This plates are incubated at 35-37c for 18 -72 hrs. then the plates are observed for any colonies.

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Media	Description of colonies.
BGA	Small transparent, colourless or pink or white opaque surrounted by pink or red zone.
BSA	Black and green colonies.
DCA	Yellowish colonies.

SECONDRY TEST:

If colony confirms to the description then it is subculture onto triple suger iron Agar(TSIA) slants by inoculating the surface of slop first then stabing the slants are incubated at 35-37c for 18-72hrs. the presence of salmonella is confirmed if in the deep culture but not the surface, there is a change of colour from red to yellow and usually formation of acid and gas in stab culture with or without production of H_2S in the agar.

1. Dextrose fermentation and slight H_2S production.
2. Dextrose fermentation and aboundant H_2S production.
3. Uninoculated control.

- PSEUDOMONAS AERUGINOSA :-

1. It is Gram negative bacilli,nonsporing, aerobic bacteria.
2. It is Gram negative slender type bacteria,which is of 1.5 – 3 micron.
3. It is motile by polar flagella ,non capsulated bacteria.
4. The media is used cetrimide Agar.
5. It is caused infection wounds , eye infection ,urinary infection.

TEST:-

1. Aseptically transfer 10ml of water to 90ml of soyabean casein digest medium.
2. Incubated at 35 -37c for 18 – 48 hrs.

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PRIMARY TEST:-

The enrichment culture is streaked onto cetrimide ager (CA) and incubated at 35- 37c for 18 - 72 hrs. if greenish flurescence under UV light (254nm) is obtained then secondry test is , plates carried out called pigment test and oxidase test.

Table:

Serial number.	Selective media.	Charactristic colonial morphological.	Flurescene in UV light.
1.	Pseudomonas ager medium for detection of flurescent.	Generally colourless to greenish.	Yellowish.
2.	Pseudomonas ager medium for detection of pyocynin.	Generally Greenish.	Blue.
3.	Cetrimide ager.	Generally greenish.	Greenish.

SECONDRY TEST:-

Pigment test :-

With inoculating loop, the suspected colonies from the syrfaces of cetrimide ager are streaked on pseudomonas ager medium for detection of flurescene and pseudomonas ager medium for detection of pyocyanin and contained in petidishes and incubated at 35- 37c for 18 – 72 hrs.

After incubation, streaked surfaces are examined under the UV light to determine the colonies are having the carecteristic.

Oxidase test:-

The suspected colonies are smeared on the oxidase test strips (N,N dimethyl-p-phenylene diamine oxalate) the test is positive, if purple colour is produced with in 5-10 seconds, if not or produced later on indicates the absence of pseudomonas.

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➢ STAPHYLOCOCCUS AUREUS:-

1. The word staphyle derived from greek word meaning bunch of grapes.
2. It is Gram positive bacteria.
3. It is spherical cocci.
4. It is non motile, found singly in pairs.
5. It is facultative anaerobic bacteria.
6. The media is used is manitol salt Agar.

TEST:-

Aseptically transfer 10ml of water to 90ml of soyabean casein digest medium.

Incubated at 35-37c for 18 – 48 hrs.

PRIMARY TEST:-

The enrichment culture is streaked on vogel-johnson agar and manintol salt agar, Baired parker agar and incubated at 35- 37c for 18-72hrs. if the colonies observed and confirmed the description given below, indicates the presence of staphylococcus aureus.

Table:-

Serial no.	Medium.	Colony characteristic.	Gram stain.
1.	VJA	Black colonies surrounded by yellow zone.	Positive cocci in cluters.
2.	MSA	Yellow colonies with yellow zone.	Positive cocci in clusters.
3.	BPA	Black shiny, surrounded by clear zone.	Positive cocci in cluster.

Secondry test:-

Suspescted colonies are transferred to the tube containing 5ml additives plasma (rabbit/ horse) with or without additives and incubated on water bath at 37c. the tubes examined for 3 hrs. if it

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coagulates, then it shows presence of S.aureus but if no coagulation is observed then it shows the absence of staphylococcus aureus**.**

Test for total bacterial count:-

Sample preparation:-

10.0ml sample into 90.0ml buffered sodium chloride peptone solution.[pH- 7.0]. in case of lumpy material nature it is kept at 30~35c for 30 minutes.

Test for total fungal count:-

- Same procedure is used.
- Instead of SA,SD medium is used.
- Plates incubated at 20-25c for 5 days.

Membrane filter method:-

The MFT is valuble laboratory procedure for detection and enumeration of coliforms bacteria in large volume of water sample with low coliform densities. This method is described as one with a very high degree of sensitivity. The advantage of this method is it's ability to concentrate low number of coliforms bacteria in large volume sample (e.g.100 ml samples) and technique, with modification has been adopted for many micro-biological procedure other than water examination.

Result :-

Observation:-

Test for total viable count:-

Perticulars	Name of medium.	Incubation condition.	Plate (cfu).	Result= no. of colonies * dilution factors.	Remarks.

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Total bacterial count.	SA	30-35c for 5 days.	ND	<10cfu/gm.	-ve
Total fungal count.	SD.	20-25c for 5 days.	ND	<10cfu/gm.	-ve.

Test for pathogen:

Sample preparation table result:

Enrichment:

Name of organism.	Sample preparation.	Incubation condition.	Remark.
E.coli . Salmonella s.aueus. P.aeruginosa.	10ml to 90ml SCDM.	35-37cfor 18-48hrs.	Growth observed.

Primary test:

Name of organism.	Inoculation	Incubation condition	Characteristic growth.	Remark.
E.coli.	1ml enrichment culture>>1ml MB	40-45c for 18-24 hrs.	Acid and gas production.	No growth observed.

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salmonella	1ml enrichment culture>>10ml TB and SB.	41-45c for 18-24 hrs.		No growth
P.aeruginosa	Streak enrichment culture on CA.	35-37c for 24 hrs.	Greenish colonies.	No growth.
S. aureus.	Streak enrichment on MSA.	35-37c for 24hrs.	Yellow colonies.	No growth.

Test for membrane filter technique:-

Medium inoculated	Temperature of incubation	Duration of incubation	Colony morphology	No.of organism/100ml	inference
Drinking water 100ml	37c	24 hours	Pink rods	4/100ml	E.coli positive.
Sewage water 100ml	37c	24 hours	Pink rods	uncountable	E.coli present not potable.

Conclusion:

From the above study, it can be concluded finally result are observed on the basis of their limit.

Since, the pharmaceutical of various dosage fron which the human for certain kinds of aliments to treat the dead fully diseases' ultimately use. Therefore the test should performed in all the dosage from to ensure that the formulation is free from any microorganism and it also ensure that it will not going to harm body as concern to the microorganism.

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