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Detection of staphylococcus aureus in various clinical samples and water sample and its remedial measures

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Abstract – *Staphylococcus aureus is a bacteria responsible for human infection, and it is highly infectious and resistant to antibiotics. Staphylococcus aureus bacteria give rise to simple skin wound infections to life threatening diseases. Even the presence of this bacterium in drinking water is also a prime concern because of its potential to cause water contamination and human infection. Staphylococcus bacteria also have the ability to cause food poisoning. The major problem about this bacterium is its resistance to antibiotics. MRSA and MSSA are the two types of staphylococcus aureus mostly found. MRSA is methicillin resistant staphylococcus aureus and MSSA is methicillin sensitive staphylococcus aureus. This gram positive bacterium enters the water when it gets highly polluted due to runoffs and fecal matters of human beings and animals. This project deals with detection of presence of s. aureus in various clinical and water sample in order to determine the suitable antibiotic as well the disinfectant to eliminate them from water body.*

***Key Words*: Staphylococcus aureus, bacteria, MRSA, MSSA, disinfectant, antibiotics**

1. INTRODUCTION

Staphylococcus aureus is round shaped, gram positive bacteria that can be normally found in the upper respiratory tract and on the skin. The bacteria staphylococcus aureus is one such harmful bacterium which has the ability to infect human beings, act as a food poisoning agent when present in food and also has the potential to contaminate water when present in them.

Polluted water contains e-coli normally in them. Similarly staphylococcus aureus can also be seen in polluted water. Presence of these bacteria in water is quite common during rainy season.

Presence of staphylococcus aureus can be seen in several medical samples such as blood, stool, throat swab, nose swab, urine.

Hence the detection of these bacteria at the earliest is very necessary to cure diseases and infections caused by them and also to take necessary remedial measures to eliminate the presence in water sources to make them fit for use.

Staphylococcus aureus is of two types: MRSA and MSSA.

Methicillin resistant staphylococcus aureus or MRSA is the staphylococcus aureus bacterium that is resistant to methicillin antibiotics. Infection caused by MRSA is very difficult to treat since it is highly resistant to antibiotics.

MSSA or Methicillin sensitive staphylococcus aureus is the staph bacteria which is sensitive to methicillin and other antibiotics. As a result diseases caused by MSSA can be easily cured. Compared to MRSA, MSSA is noninfectious.

Table -1: Comparison between MRSA and MSSA

Aspect	MRSA	MSSA
Definition	staphylococcus aureus that are methicillin resistant	staphylococcus aureus that are susceptible to methicillin
Effective response to antibiotic treatment	Resistant to many antibiotic	Sensitive to antibiotic treatment
Bacterial response	Resistant to methicillin	Sensitive to methicillin
Virulence	Highly infectious	Less infectious
Mortality rate	High	Low
Preferred treatment	Only vancomycin	Antibiotics such as cephalexin, amoxylin etc.

1.1 Contamination of water body by S. aureus

Staphylococcus aureus is present everywhere around us. Spread of these bacteria is common from hospitals, clinics, untidy surface etc. These bacteria are not only present in clinical samples but also in water.

Staphylococcus aureus is present in polluted water. Its presence is similar to that of e-coli. Staphylococcus aureus can be seen in water especially during rainy season.

Waterbody gets contaminated with staphylococcus aureus bacteria when the water is highly polluted with:

- Feces from infected humans and animals
- Excess surface runoff
- Sewage overflows

- Pollution and dumping of wastes
- Agricultural runoff
- Stagnation of excessive chemicals

Waterbodies such as wells, ponds etc. are highly prone to contamination by these bacteria due to less turbulence and flow of water. Even private wells during rainy season sometimes show the presence of staphylococcus aureus bacteria due to excessive surface runoff from different areas.

Since staphylococcus aureus can easily enter the water bodies due to contamination of water and also during heavy rainy season it is very vital to disinfect the water source to make the water fit for use.

Disinfection is the process of killing undesirable bacteria. Disinfectants are the substance used to carry out the processes which are basically chemicals. Quantity of disinfectant used is usually expressed in mg/l or ppm. One mg/l equals one ppm.

There are several methods of disinfection. Disinfection by bleaching powder, hydrogen peroxide and sodium hypochlorite are adopted here.

Bleaching powder is a pale yellowish powder with smell of chlorine which is a combination of lime and chlorine. It usually consists of more than 33% chlorine content. This chlorine content is responsible for the disinfection action.

The most effective and commonly adopted method of disinfecting well is to use bleaching powder. To disinfect a well nearly 100 to 200 gm of bleaching powder is required. Even if the well is highly polluted 200gm of bleaching powder is far enough, the optimum dosage of the bleaching powder.

Disinfection by sodium hypochlorite is also another chlorination method. It is also known as bleach. It is a yellowish solution which is very effective in disinfecting water source. It has a strong smell. It is sometimes used to remove odour as well.

Disinfection by hydrogen peroxide is another technique that can be adopted for well disinfection. It is a colourless liquid easily available in market. Its dosage rate is as 1 part of disinfectant to 2 parts of water sample.

2. METHODOLOGY

Since staphylococcus aureus bacteria are virulent, its presence should be detected at the earliest to take necessary preventive measures. Staphylococcus aureus are seen in clinical samples as well as in polluted water. In order to determine the suitable antibiotics for prescription purpose, various samples must be collected from the infected patients and it must be cultured by applying suitable medium to detect the presence of the bacteria.

Similarly suspicious water samples must also be collected in order to determine the presence of staphylococcus aureus bacteria and to analyze the suitable preventive measures. Following are the steps carried out to precede the project:

1. Sample collection
    - Water sample
    - clinical samples

2. Culturing

3. Catalase test and Coagulase test

4. Application of remedial measures

2.1 Sample collection

15 clinical samples were collected from hospitals from different patients which include samples of skin wound, pus, stool, urine, nasal swab and throat swab.

Water sample was collected from a public well located in a densely populated colony.

Both the clinical and water sample is then tested to detect the presence of staphylococcus aureus in them.

2.2 Sample Culturing

The staphylococcus aureus is to be isolated from the different clinical samples, prepare smear and gram staining is carried out to determine whether it is gram positive or negative. The samples were inoculated into sterile MacConkey agar and blood agar. Inoculated plates are incubated at 37 degree celcius for 24 hours. In blood agar the medium is prepared by adding sterile blood to sterile nutrient agar medium, autoclaved and cooled to 45 degree celcius and then appropriate amount of blood is poured. The concentration of blood used is 8%.

In MacConkey agar the medium is prepared by suspending the components like peptone, agar, bile salt, lactose, and neutral red in distilled water. Mix well, sterilize and autoclave at 121 degree celsius for 15 minutes, cool to 45-50 degree celsius, mix well and dispense onto plates. The plates are then left to solidify and dry. Bacterial colony will develop on the slide when sufficient nutrient medium is provided. Gram staining procedure is then carried out with each type of colony obtained in blood agar and MacConkey agar. If the bacteria are gram positive, catalase test and coagulase test are performed to detect the organism.

Gram staining procedure is carried out to determine whether the bacteria is gram positive or gram negative. The

procedure is based on the reaction between peptidoglycan in the cell walls of some bacteria. In the beginning the bacteria is stained with crystal violet where both the positive and negative bacteria will stain to purple colour. It is then treated with Gram's iodine (iodine and potassium iodide), a mordant or fixative. The gram-positive cells would form a crystal violet-iodine complex. Alcohol or acetone is then applied to decolorize the cells. Gram-negative bacteria will become colourless, while only some of the colour is removed from gram-positive cells. The last step involves the usage of a counterstain (safranin) to colour the bacteria pink. Both gram-positive and negative bacteria pick up the pink stain. If the procedure is performed well, gram-positive bacteria will be purple in colour while gram-negative bacteria will be pink.

2.3 Catalase Test and Coagulase Test

Catalase test is used to demonstrate the presence of an enzyme named catalase that catalyzes the release of oxygen from H_2O_2. The test is performed on a slide. A drop of 3% hydrogen peroxide is placed on the slide and an isolated colony is picked up with a sterile stick and placed on this slide containing 3% hydrogen peroxide. It is then observed immediately for active bubbling.

Coagulase test is the most important test to identify or confirm a bacteria whether it is staphylococcus aureus or not. It can be carried out in two ways, either as slide coagulase test (SCT) or Tube coagulase test (TCT). Coagulase is an enzyme like protein which has the ability to clot plasma by converting fibrinogen to fibrin.

In slide test, over a glass slide a drop of normal saline is placed on each end. Emulsify a colony of the test organism in each of the drops to make a thick suspension. Add plasma to one of the suspension and the other is taken as control, mix gently. Observe for clumping of the organism within 10 seconds.

To carry out tube coagulase test, the plasma is diluted as 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8 ml of saline). Pipette out 0.5 ml each into three test tubes labelled as T (Test), P (Positive control) and N (Negative control). Test is 18-24 hr broth culture consisting of 5 drops of test organism whereas positive control contains 5 drops of S. aureus broth culture and negative control contains 5 drops of sterile broth. Incubate the three tubes for 35-37^{0}C. It is then examined for clotting after 1 hr.

2.4 Remedial Measures

After the detection of staphylococcus aureus in clinical samples as well as water sample, it is necessary to apply suitable remedial measures in order to kill the bacteria present in them.

In the case of clinical samples, the detection of staph bacteria helps the doctor to prescribe the suitable antibiotics to the infected person which helps to cure the disease at the earliest.

In the case of water sample, after the detection of the presence of the bacteria, disinfection process is carried out to make the well water fit for use. Two types of disinfection methods are available to disinfect staph bacteria. They are:

- Disinfection using chemicals
- Disinfection using natural agents

2.4.1 Disinfection using chemicals

In this method, three chemicals are chosen to disinfect staphylococcus aureus bacteria. They are:

i. Disinfection using bleaching powder

The well sample is treated with bleaching powder to detect whether it can disinfect the sample or not.

Procedure:

- Take 1000ml of the water sample.
- Take 5 g of bleaching powder
- Make a thick paste using water free from impurities
- Add 100 ml of water to the paste
- Stir the mixture thoroughly
- Allow the solution to rest
- A supernatant solution forms leaving behind a residue.
- Pour this solution to the water sample.
- Test for the presence or absence of S. aureus

Fig-1: Bleaching powder

Fig-2: Bleaching powder diluted and supernatant liquid formed

ii. Disinfection by sodium hypochlorite (NaOCl)

Procedure:

- Take 1000 ml of the water sample
- 100ml of NaOCl solution is added to the sample taken
- 5% concentrated sodium hypochlorite is used.
- Shake well the disinfectant and water sample
- Test for the presence /absence of s. aureus bacteria.

Fig-3: NaOCl solution

iii. Disinfection by hydrogen peroxide

Procedure:

- Take 1000 ml water sample
- Take 500ml of H_2O_2 solution
- Commonly available hydrogen peroxide is 3% w/v which is stable and effective
- 1 part of disinfectant should be added to 2 parts of water sample, the dosage rate of hydrogen peroxide.
- Mix thoroughly the sample and disinfectant.
- Allow to rest for more than 15 mins.
- Test for the presence /absence of staph bacteria.

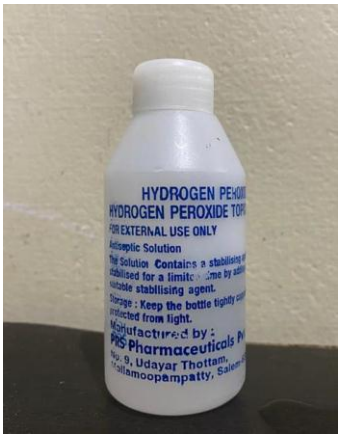

Fig-4: H_2O_2 solution

2.4.2 Disinfection by natural agents

Staphylococcus aureus can be disinfected not only by chemicals but also by natural agents such as turmeric, ginger and garlic. Though usages of chemical disinfectants are effective, these natural items also have the potential to kill staph bacteria.

i. Turmeric:

Curcumin present in turmeric is the main factor that inhibits the bacterial growth. They are well known for their antibacterial property. Here, turmeric in powdered form is used as disinfectant to kill staph bacteria

Procedure:

- 1000 ml water sample is taken
- 100 g of turmeric powder is made into fine paste.
- Make sure there are no lumps.
- Mix the fine paste thoroughly with the water sample.
- Allow to rest for few hours.
- Test for S. aureus

ii. Ginger

Ginger is one of the most effective antibiotics that can destroy bacteria. Ginger also has the ability to kill staphylococcus bacteria.

Procedure:

- Take 1000 ml of water sample.
- Finely grind 250 g of ginger to a paste.
- Add the paste to the water sample and mix thoroughly

- Allow to rest for more than 2 hours
- Test for the presence of staphylococcus bacteria.

iii. Garlic

Garlic is also well known for its antimicrobial property. From ancient times itself garlic has been used to cure several infections and kill bacteria.

Procedure:

- Take 1000 ml of water sample.
- Finely grind 350g of garlic to a paste.
- Add it to the water sample.
- Shake well to ensure thorough mixing.
- Allow to rest for more than 2 hours
- Test for the presence of staphylococcus bacteria.

3. RESULTS AND DISCUSSION

Various clinical samples and the water sample were tested to detect the presence of staphylococcus aureus in them. The results obtained are as:

Among the 15 clinical samples collected, 12 samples were methicillin sensitive staphylococcus aureus (MSSA) and 3 samples were Methicillin resistant staphylococcus aureus (MRSA) strain.

Similarly, water sample collected from well also showed the presence of staphylococcus aureus in it.

After inoculation and incubation of the samples in blood agar and MacConkey agar the colonies appeared as:

- On blood agar: β hemolytic colonies
- On MacConkey agar: lactose fermenting colonies (pinkish colour)

Lactose fermenting and β hemolytic colonies are the characteristic features of staphylococcus aureus.

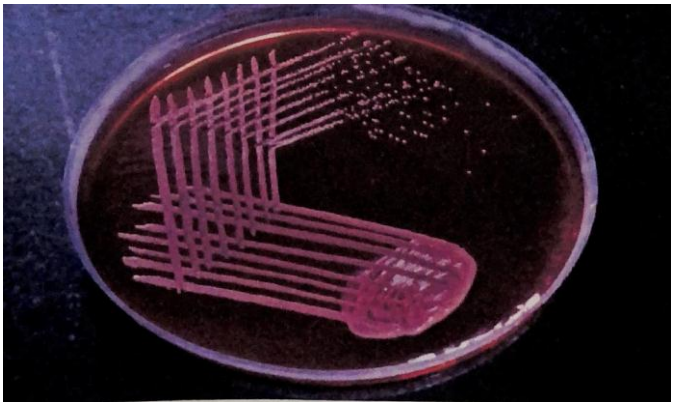

Fig-5: Colony morphology of staphylococcus aureus in MacConkey agar

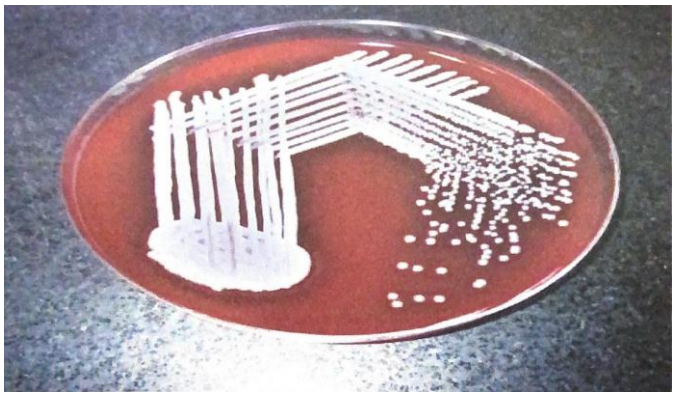

Fig-6: Colony morphology of staphylococcus aureus in blood agar

While gram staining Gram positive bacteria will stain purple colour when crystal violet or primary stain is applied. It still retains the colour of violet blue when safranin is added even after decolorizer is applied.

The samples collected turned out to exhibit violet blue colour at the end of procedure which indicates that the samples are gram positive cocci.

The gram positive cocci are further analyzed to confirm whether organism present in the sample collected is

In the catalase test the slide containing well isolated colony and hydrogen peroxide was observed immediately for active bubbling and is declared as positive.

In slide coagulase test, Clumping of the organism within 10 minutes must be observed within the suspension. The organism clumped up, hence read as coagulase positive staphylococci.

Similarly, in the tube coagulase test, the organism along with saline and plasma is observed to clump. Hence it is regarded as coagulase positive.

3.1 Remedial Measures

In the case of clinical samples, they are cultured in hospitals and clinics to determine which antibiotic can be prescribed to cure the diseases caused by the concerned bacterium.

An antibiotic is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of infections.

After culturing, antibiotic susceptibility is carried out for the colony which helps to determine which antibiotic at what dosage must be given to patient in order to cure the concerned disease at the earliest.

Antibiotics such as penicillin, ampicillin, amoxyclav, gentamicin etc can be used to cure MSSA variant. Whereas vancomycin is the only antibiotic that is capable to kill MRSA variant because it is highly resistant to all other antibiotics.

The water sample collected from public well was also cultured to detect the presence of s. aureus in them. The sample collected showed the presence for the same.

Hence it is now necessary to take remedial measures to make the water fit for use. Hence disinfection is the solution to kill the organism present in the sample. Disinfection by chemicals as well as using natural agents can be used as a remedial measure to eliminate the bacteria in water to make it again fit for human consumption. The results after treating the water samples with each disinfectant are given below.

Table -2: Disinfectants and the required quantity

Name Of Disinfectant	Water Sample	Disinfectant Quantity	Action Detected
Disinfection By Chemicals			
Bleaching Powder	1000 ml	5 gm	Disinfected
Sodium Hypochlorite	1000 ml	100 ml	Disinfected
Hydrogen Peroxide	1000 ml	500 ml	Disinfected
Disinfection By Natural Items			
Turmeric	1000 ml	100 g	Disinfected
Ginger	1000 ml	250 g	Disinfected
Garlic	1000 ml	350 g	Disinfected

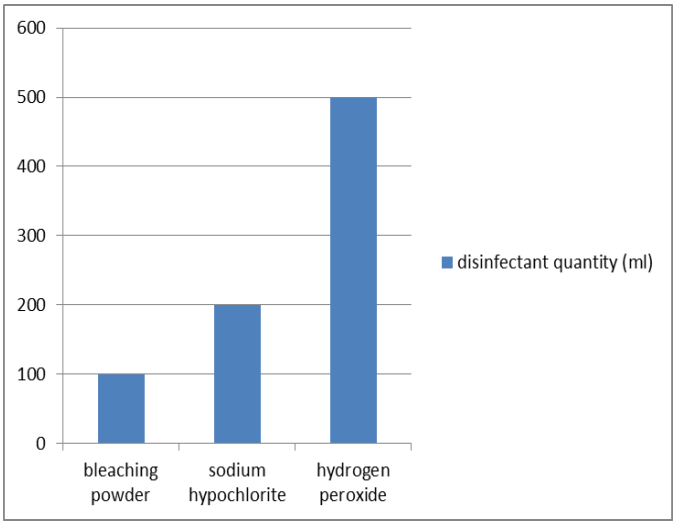

Chart -1: Chemical disinfectant and their quantity

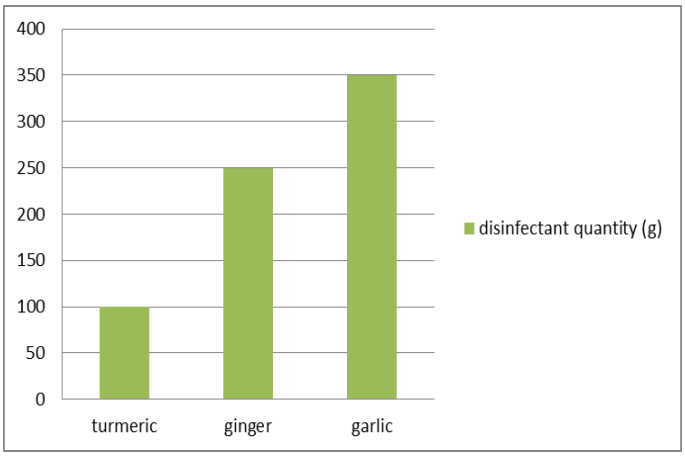

Chart -2: Natural disinfectant and their quantity

From the above two charts it is very clear that bleaching powder and turmeric are the disinfectants that are used in least quantity. The other disinfectants are also effective but these two are used in lesser amount compared to other disinfectants and the desired results are attained.

In the case of disinfectant with chemicals all the three used, bleaching powder, sodium hypochlorite or bleach and hydrogen peroxide are effective disinfectants. But the most widely used disinfectant for well water disinfection is bleaching powder. This is because whatever the contamination level may be the optimum dosage for bleaching powder ranges between 100 to 200 mg whereas the dosage of the other two disinfectants varies with the intensity of contamination. If the well water is highly polluted the amount of disinfectant used also increases.

Hence the most effective and widely used disinfectant for well water is bleaching powder.

4. CONCLUSIONS

Staphylococcus aureus is a virulent gram positive bacterium that is capable of causing diseases ranging from simple infections to death causing diseases in human beings. Apart from their infectious capability, these bacteria also have the ability to cause food poisoning when present in food matter.

These bacteria can easily be seen in polluted water bodies such as well, pond etc. where there is high pollution and different kind of runoffs reaching these water sources. The presence of this bacteria is common during rainy season due to low turbulence and lack of flow in well and pond. Consumption of this water causes diseases and infections to human beings.

As a result detection of these bacteria at the earliest is important to detect the presence of these bacteria in water as well as in humans to adopt necessary measures. Detection of staphylococcus aureus bacteria in clinical samples helps to determine the presence or absence of the bacterium in the samples collected such as stool, urine, throat swab, nose swab, blood etc. and also helps to determine whether the variant is MSSA or MRSA and helps to find out the suitable antibiotic to cure them.

Detection of these gram positive bacteria in water sources helps to eliminate its presence and makes the water fit for use.

After the detection of staph bacteria in water, disinfection must be carried out to make the sample free of this organism.

Either disinfection by chemicals or disinfection by natural agents can be used.

Disinfection by chemicals includes the usage of bleaching powder, sodium hypochlorite solution and hydrogen peroxide. All the three disinfectants are effective at eliminating organisms present in the well water. But the most and widely used disinfectant is bleaching powder. This is because it is cheap and easily available in the market. At the same time a smaller quantity of disinfectant is capable to remove the entire contamination. Even for highest contamination level in the well water about 100 to 200 g of bleaching powder can disinfect the water enough. Whereas in the case of hydrogen peroxide and sodium hypochlorite the dosage limit varies with contamination level. Hence when contamination increases the amount of disinfectant used also increases. This limits the usage of these disinfectants. Therefore usage of bleaching powder as disinfectant for well water turns out to be economical.

Disinfection by natural agents includes the usage of turmeric, garlic and ginger. All the three are known for their antimicrobial activity since ancient times. Here quantity of turmeric used is less for disinfection compared to ginger and garlic. Though these products do not pose any harmful effects to the consumers they are not widely used especially for disinfection of well water since a large quantity of turmeric, ginger and garlic would be required which turns out to be impracticable and costly.

Hence for the disinfection of well water we most commonly use bleaching powder because of its easy availability, cheapness, efficiency and limited dosage.

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