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A STUDY ON DRUG SENSITIVITY OF *Staphylococcus aureus* ISOLATED FROM WASTE WATER OF HOSPITAL ENVIROMENT

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ABSTRACT

The aim of this study to investigate the water sample to find out how many strains of *Staphylococcus aureus* were contaminating. Different antibiotic means different strains of isolate. Medically important pathogens of *Staphylococcus aureus* strains were isolated from water sample and examined. Manitol salt agar was used for the isolation of *Staphylococci* and comfirmation of *S.aureus* by means of Gram staining technique. Antibiotic susceptibility testing was performed by disc diffusion method. Antibiotic susceptibility tests indicated that four isolated were present in the water sample. Out of these four isolated, two were found to be multiple drug resistant. These two strains are examined by minimal inhibitory concentration of vancomycin. Antibiogram as a typing method could be used to differentiate one isolated from another.

KEYWORDS: Important pathogens, antibiotic susceptibility, multiple drug resistant.

1. INTRODUCTION

The suitability of a water supply is determined by four types of analysis:

(1) The chemical analysis determines total solids, hardness, etc… and detects any harmful chemical ingredients such as poisonous lead or zinc salts:
(2) The physical examination determines if the water has any objectionable, salinity, turbidity, colour, taste of odour:
(3) The biological analysis detects algae, fungi, protozoa, nematodes worms and larva of aquatic insects:
(4) The bacteriological analysis is the most valuable examination and is vital in preventing epidemics as a result of water pollution.[4]

Staphylococcus aureus can give rise to many serious skin infections with consequent systemic infections. MRSA was emerging in hospital environments and also in the community. Thus *Staphylococcus aureus* strains were isolated and drug sensitivity test was used to characterize the isolates to show that this typing method can differentiate each isolated from another. This will be the valuable data for regarding the contamination of water.[5]

2. MATERIALS AND METHODS

Waste water sample and culture media such as nutrient agar, manitol salt agar, Muller Hinton broth were purchased from Australia Medical Diagnostics (AMD, company, Yangon Myanmar).

2.1 Sample collections and storage

Water sample is taken and stored at 4˚C before examination.

2.2 Isolation of *Staphylococcus aureus*from waste water sample

First, the water sample was centrifuged and the sediment was cultured on nutrient agar with the added of 7% NaCl. Select twelve of milky convex colonies and studied by Gram's stain. The gram positive cocci with grape like structure was obtained. Then this colony was subculture on Manitol salt agar Fig-1 and examined by microscope and motality test. The colony *Staphylococcus aureus* was noted to ferment manitol and was no-motile. Figure 1.[2]

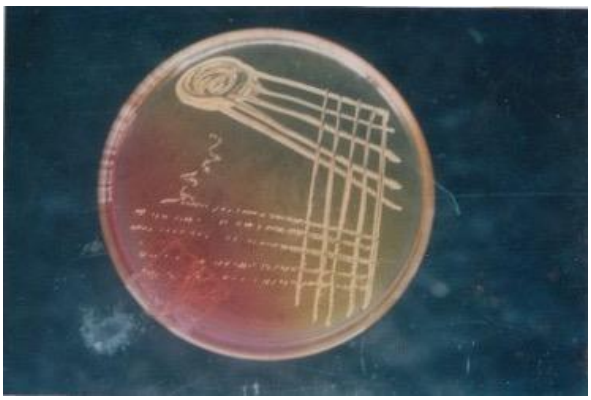

Figure 1: *Staphylococcus aureus* on Mannitol Salt Agar.

2.3 Disc diffusion method

One of four similar colonies. On the stock culture were inoculated into Tryptone Soya Broth (TSB) and incubated at 37˚C for 2-3hours till light to moderate turbidity develop This suspension was streaked on Muller Hinton agar. Then it was dried at room temperature for 5 minutes with lid in the disc at least 24mm apart. Then the plates were incubated at 35˚C for 24 hours. The incubation zone size were examined and interpreted according NCCLS guideline.Figure 2 and Table 1.[7]

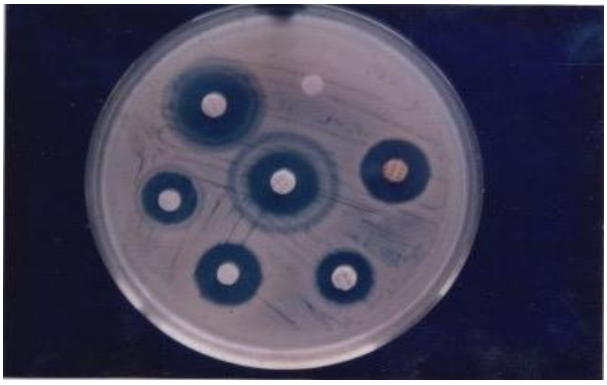

Figure 2: Disc Diffusion Plate of *Staphylococcus aureus* Isolates to Seven Antibiotics.

Table 1: Antimicrobial Susceptibility Tests of *Staphylococcus aureus* Isolates to Various Antibiotics.

No	Antibiotic	Concentration (mcg)	Antibiotic Sensitivity Pattern				Zone Size				Zon Size interpretation		
			C1	C2	C3	C4	C1 (mm)	C2 (mm)	C3 (mm)	C4 (mm)	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
1	Ampicillin	30	+	+	+	-	-	-	-	17	15 ≤	12-14	11 ≥
2	Kanamycin	30	-	-	-	-	22	24	25	24	18 ≤	14-17	13 ≥
3	Streptomycin	10	-	-	-	-	14	19	16	21	15≤	12-14	11 ≥
4	Choramphenicol	30	+	I	+	I	-	26	17	26	29≤	26-28	25 ≥
5	Vanvomycin	300	-	-	-	-	14	14	16	16	12≤	10-11	9 ≥
6	Tetracycline	30	+	-	I	-	-	19	18	24	19≤	15-18	14 ≥
7	Erythromycin	15	I	-	I	I	20	23	19	21	23≤	14-22	13≥

(+) ⟶ Resistant (-) ⟶ Resistant (I) ⟶ Resistant

2.4 Minimal inhibitory concentration dilution technique

Select four or five similar colonies. Transfer these colonies in turn to a test tube containing about 5mm of suitable liquid medium. Incubate the tube at 35˚C - 37˚C, for about 4hours to produce organism suspension with moderate colonies cloudiness. At that point the inoculum density of the suspension should be compared with McFarland Equivalence Turbidity Standard to obtain 5 × 10^5 colony forming units. Prepare Mueller – Hinton Broth and 100µg/ml concentration of antibiotic label 10 sterile 13 × 100mm test tubes, 1,2,3,…… etc, and pipette 0.5 ml of Muller Hinton broth into each test tube. Into tube pipette 0.5ml of the antibiotic solution (100µg/ml) and mix well. Transfer 0.5 ml of tube 1 to tube 2 and mix. Then transfer 0.5ml of tube 2 to tube 3 and continue this process until reach tube 8, tube 9 will not receive 0.5ml from tube 8 and thus serve as a control. Take 0.5ml from tube 8 and discard into the sink. Make sure to use a separate pipette for each transfer. This two fold serial dilution is standardized inoculum (5 × 10^5cfµ/ml). Incubate all tubes at 35˚C for 18hours. After incubation, visually determine which tube show no growth. The lowest concentration of antimicrobial that results is no visible growth is MIC.Table 2.[6]

Table 2: Minimal Inhibitory Concentration.
(a)Microorganisms used Staphylococcus aureus (C_1)
Antimicrobial used Vancomycin

Tube	Concentration of Antimicrobial (µg/ml)	Growth (G) or No. Growth (NG)
1	50.0	NG
2	25.0	NG
3	12.5	NG
4	6.25	NG
5	3.12	NG
6	1.56	G
7	0.78	G
8	0.39	G
9(control)	0.00	G

MIC= 3.125µg/ml^{-1}

(b) Microorganism used *Staphylococcus aureus*(C_2)
Antimicrobial used Vancomycin

Tube	Concentration of Antimicrobial (µg/ml)	Growth (G) or No. Growth (NG)
1	50.0	NG
2	25.0	NG
3	12.5	NG
4	6.25	NG
5	3.12	NG
6	1.56	NG
7	0.78	NG
8	0.39	G
9(control)	0.00	G

MIC = $0.78\mu g/ml^{-1}$

3. RESULTS AND DISCUSSION

The water sample was centrifuged and culture on Nutrient Agar with added 7% NaCl. Nutrient agar was found to be gram positive cocci of grape like structure. Subculture was done on Manitol salt agar. *Staphyloccusaureus*, which is fermentative on manitol salt agar were observed. It was confirmed by Gram's stain and direct microscopic examination

Twelve single colonies on Manitol salt agar were subjected to Antibiotic susceptibility. Test by disc diffusion method with the aim to investigate how many isolates are there in the water sample. Four isolates were observed with different antibiotic susceptibility pattern. Table 1 gives more detail on antibiotic susceptibility. All strains are similar in sensitive to kanamycin, streptomycin and vancomycin

Among these four strains, C_1 and C_2 were studied by dilution for minimal inhibitory concentration of vancomycin because they had 14mm inhibition zone size. The MIC value of C_1 is 3.125µg/ml and C_2 is 0.78µg/ml. These results were shown in Table 2.

The medically important bacteria of *Staphylococcus aureus* was isolated from waste water sample and examined the various strains of *S.aureus* by antibiotic susceptibility pattern of seven antibiotics.

As a general rule *S.aureus* strains are highly sensitive to most of the antibiotics used in therapy. Growth of these strains is prevented by 0.02-0.05µg/ml of benzyl penicillin, 0.5-2 µg/ml of methicillin, 0.5 µg/ml of streptomycin, 1.5-10µg/ml of chloramphenicol, 0.1-1.0 µg/ml of tetracyclines and 0.25 µg/ml of erythromycin dependent to a certain extent on the method of test.[3]

In the hospital environment, and to a lesser extent in general community, the proportion of strains isolated from carriers and lesions that are resistant to antibiotics used in therapy has increased, roughly in proportion to the amount of these substances used. Thus in many hospital today over 80 percent, of strains isolated are penicillin resistant, 50 percent are resistant to Streptomycin, 25-50 percent are tetracycline resistant, and 5-15 percent are resistant to Chloramphenicol. Among general community about 20-30 percent of strains are penicillin resistant, and much small proportions are resistant to the other antibiotics (Aricher 1995).[1]

The aim of this study was investigated the water sample to find out how many strains of *S.aureus* were contaminating.. Different antibiotic susceptibility means different strains of isolates.

According to the date mentioned, four *S. aureus* were isolated. Out of which 2 isolates were found to be multi drug resistant. Regarding vancomycin sensitivity, the result of disc diffusion method showed zone size of 14 mm in strains $C_1$and C_2 respectively. Vancomycine intermediate *S.aureus* and vancomycine resistant *S.auresus* are important pathogens. To be sure, that these three isolates were sensitive to vancomycin, tube dilution method was proceeded for these isolates and found to be vancomycin sensitive. As a conclusion to tube dilution test all isolates were found to be vancomycin sensitive.

The data obtained in this study could give valuable information on bacteriological contaminating level of waste water sample and presence of highly pathogenic *S.aureus* strains.

4. CONCLUSION

It was sure that the water was contaminated with intestinal pathogens and skin pathogen. Out of these pathogens, *S.aureus* an important skin as well as systemic pathogen was studied. Isolation of *S.aureus* was done and isolates were studied by antibiogram to investigate whether there was presence of numerous isolates or not Different antibiotic patterns indicated different isolates, two out of four isolates were multi – drug resistant.

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