Screening of Multi-drug Resistance of *S. aureus*(*Rosenbach.*) from Hospital Waste-A Case Study.

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Abstract

The study was conducted during the session of 2012-2013, and aimed to collect the clinical and hospital waste samples from different sites, their isolation and identification of *Staphylococci aureus*(*Rosenbach.*), and antibiogram of Multi-drug Resistant *Staphylococci aureus*(*Rosenbach.*). Similarly, *t*o develop the method and to reduce the rise in spread of diseases associated with hospital waste. The samples were collected in 100 ml of sterilized bottle and were transported to laboratory for analysis within 2-hours and then isolation of *Staphylococci aureus*(*Rosenbach.*), the loop full cultures were inoculated on Mannitol Salt Agar, Baired Parker Agar and Milk agar media. The pure cultures were isolated and studied for morphology and biochemical characteristics. The biochemical characteristics of all clinical isolates were studied by inoculating the cultures in Glucose, Lactose, Sucrose, and Mannitol broth respectively for IMVIC test. Cultures were inoculated in Tryptone broth, MRVP broth, or Glucose-Phosphate broth, and Simmon's citrate agar. Coagulase and catalase studies were also carried out. All cultures were studied for colony characterization and then isolates were reported to be gram positive cocci in bunches. From the present study, it was evident that, the over use of drugs, intravenous drug abuse, incorrect diagnosis, unwanted prescription, improper use of antibiotics by patients, improper use of antibiotics as livestock food additives for growth promotion which leads to mutation and thus leading to drug resistance. For healing the wounds, cuts and scrapes of skin should be kept clean and covered with a bandage.

Keywords:- *Staphylococcus aureus*(*Rosenbach.*), Multiple-Drug Resistance *Staphylococcus aureus*(*Rosenbach.*)(MRSA), Methicillin Resistance *Staphylococcus aureus*(*Rosenbach.*) (MRSA). Hospital waste, gram positive, Biochemical characteristics, Inoculation.

Introduction:

Staphylococci is recognized as a genus of spherical bacteria of the family micrococci, it is the best known species of which are universally present in very great number on mucous membranes and skin of humans, and also on some other warm-blooded animals. Two types of *Staphylococci* on the basis of pigmentation was recognised[20], The data on hazardous and on hazardous hospital waste were studied by many workers[6]. The constitutive erythromycin resistance plasmid in *Staphylococcus aureus*(*Rosenbach.*) were also studied[11].The antibiotic resistance of *S. aureus(Rosenbach.)* isolates from community acquired *S. aureus*(*Rosenbach.*) from Senegal were also reported by the scientist[9].The wide spread presence of MRSA strains informites derived from the hospital and suggested the of more precaution in clinical area to control the spread of the MRSA and amalgamation of antibiotics is an effective remedy for MRSA infections and promising approach in developing country[13]. The intrinsic resistance to β-Lactam antibiotics in *S. aureus*(*Rosenbach.*) were reported by many workers[6]. The Intrinsic resistance to lactam antibiotics in *S. aureus*(*Rosenbach.*) were also reported[6]. The reports on *Staphylococcus*(*Rosenbach.*) wound infections in a surgical ward at Lucknow were published[2,4]. The term *Staphylococcus*(*Rosenbach.*), generally employed for all the species, refers to the cell's

habit of assembling in grape like clusters, staph is a commonly used slang name for this bug. The reports on the global rise of antimicrobial resistantance of *Staphylococcus*(*Rosenbach.*) were also reported[8]. Prevention, and control of infection caused by methicillin resistance *Staphylococcus aureus*(*Rosenbach.*) (MRSA) is an important problem in a public health[13]. The infection caused by pathogenic *Staphylococcus aureus*(*Rosenbach.*) can be controlled with the help of anti-microbial agents. The estimated number of people developing a serious MRSA infection (i.e. invasive) in 2005 was about 94,360; this is higher than estimates using other methods. Approximately 18,650 persons died during a hospital stay related to serious MRSA infection. Hospital waste means all waste coming out of hospital of which around 80% are actually non-hazardous, around 15% are infectious waste and 5% are non infectious but hazardous wastes[6]).Many work published on live stalk associated MRSA (LA-MRSA) by many prominent workers [16,22]. The hospitals today is a system of increasing complexity. The great challenge is one of the co-ordination of Hospital are among the most complex organization in modern society[6].The modern hospital is a social universe with a multiplicity. The care of the patient is a master values even for those whose work seldom brings them direct contact with sick people and the patient is the hospital's client.

Materials and Methods:

Collection of samples: Samples from Hospital waste were collected bi-weekly from Wardha city (M.S). The samples were collected in 100 ml of sterilized bottle and were transported to laboratory for analysis within a period of 2-hours.

Isolation of *Staphylococcus aureus*(*Rosenbach.*): Samples were enriched in a nutrient broth fortified with 7.5% NaCl, 37^0C for 3-6 hours in an incubator and then for isolation of *S. aureus*(*Rosenbach.*), the loop full cultures were inoculated on Mannitol Salt Agar(MSA), Baired Parker Agar(BPA), and Milk agar. The pure cultures were isolated and studied for morphology and biochemical characteristics.

Screening of *Staphylococcus aureus*(*Rosenbach.*):- The morphology of all the clinical isolates were studied by performing gram staining, which is the most important differential stains used in bacteriology. The biochemical characteristics of all clinical isolates were studied by inoculating the culture in Glucose(Glu), Lactose(Lac), Sucrose(Suc) and Mannitol(Man) broth. For IMViC test cultures were inoculated in Tryptone broth, MRVP broth, or Glucose-Phosphate broth. Simmon's citrate Agar. The entire oxidase enzyme study were also carried out.

Sensitivity resistance pattern of staphylococcal isolates by Disc Diffusion (DAD) test:-

A suspension (1ml) of test organisms were thoroughly mixed with 10 ml of sterile liquid nutrient. Agar were already contains the base layer. After solidifying, the antibiotics impregnated discs were placed on the seeded agar. The plates were then incubated at 37^0C for 24 Hours. After confirmation of Multi-drug Resistance Staphylococcal isolates were subjected to sensitivity/ resistance pattern test by standard method [2,3,4].

Antibiotics Suscceptibility test:- The bacterial cultures of *S. aureus*(*Rosenbach.*), were maintained on nutrient agar at 4^0C and were sub cultured. A suspension (1ml) of *S. aureus*(*Rosenbach.*) were thoroughly mixed with 10 ml of sterile liquid nutrient agar, which already containing base layer. After solidifying the antibiotics, discs were placed on the seeded agar. The plates were then incubated at 37^0C for 24hours and the results were interpreted as sensitive, moderately sensitive, and resistant, by

employing the ‘‘zone size interpretative table’’, provided by the manufacturers of the Hi-media Laboratories Private Ltd, Mumbai (M.S).

Observation Table:

Morphological and cultural characters of Staphylococcal isolates on Mannitol Salt Agar (Table No.1).

Isolates No.	Gram Reaction	Shapes	Motility	Size	Elevation	Margin	Opacity	Consistency	Colour
Sca-1	+ve	cocci	NM	0.5-1 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-2	+ve	cocci	NM	2.9 mm	Convex	Entire	Opaque	Moist	Pink
sca-3	+ve	cocci	NM	1.0 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-4	+ve	cocci	NM	0.4 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-5	+ve	cocci	NM	0.6 mm	Convex	Irregular	Opaque	Moist	Yellow
Sca-6	+ve	cocci	NM	0.5-1 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-7	+ve	cocci	NM	3.5 mm	Convex	Entire	Opaque	Moist	Pink
Sca-8	+ve	cocci	NM	2.8 mm	Convex	Entire	Opaque	Moist	Pink
Sca-9	+ve	cocci	NM	3.5 mm	Convex	Entire	Opaque	Moist	Pink
Sca-10	+ve	cocci	NM	0.5-1 mm	Convex	Irregular	Opaque	Moist	Pink
Sca-11	+ve	cocci	NM	0.5-1 mm	Convex	Entire	Opaque	Moist	Pink
Sca-12	+ve	cocci	NM	1.2mm	Convex	Entire	Opaque	Moist	Pink
Sca-13	+ve	cocci	NM	1.5 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-14	+ve	cocci	NM	0.9 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-15	+ve	cocci	NM	1.0 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-16	+ve	cocci	NM	1.0 mm	Convex	Entire	Opaque	Moist	Yellow
Sca-17	+ve	cocci	NM	3.9-4 mm	Convex	Entire	Opaque	Moist	Pink
Sca-18	+ve	cocci	NM	3.9 mm	Convex	Regular	Opaque	Moist	Pink
Sca-19	+ve	cocci	NM	3.0 mm	Convex	Entire	Opaque	Moist	Pink
Sca-20	+ve	cocci	NM	1.0 mm	Convex	Entire	Opaque	Moist	Yellow

+ve-Positive, NM-Non motile, mm-Milimeter,

Table No. 2 Biochemical characteristics of *Staphylococcal isolates*.

Test	Biochemical test				Carbohydrate test				
S. N.	I	MR	VP	C	Glu	Lac	Man	Suc	Raf
Sca-1	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-2	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-3	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-4	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-5	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-6	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-7	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-8	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-9	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-10	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-11	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-12	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-13	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-14	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-15	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-16	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-17	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-18	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-19	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve
Sca-20	-ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve

S- Staphylo, c-coccus, a-aureus, +ve-Positive, -ve-Negative. I- Indole,

Mr-Methyl-red, VP- Voges-proskauer, C- Citrate, Glu- Glucose, Lac-Lactose,

Man- Mannitol, Suc-Sucrose, Raff- Raffinose.

Table No. 3. Antibacterial Sensitivity Pattern of Staphylococcus isolates.

S. N.	M 5 µg	V 30 µg	K 30 µg	T 30 µg	A 10 µg	P 10 µg	C 30 µg	E 10 µg
Sca1	R	R	R	S	R	R	S	S
Sca2	R	R	R	R	S	R	S	S
Sca3	R	R	R	S	S	R	S	S
Sca4	R	R	R	S	C	R	S	S
Sca5	R	R	R	S	S	R	S	S
Sca6	R	R	R	R	R	R	S	S
Sca7	R	R	R	S	S	R	S	S
Sca8	R	R	R	S	S	R	S	R
Sca9	R	R	R	S	S	R	S	S
Sc10	R	R	R	S	S	R	S	S
Sc11	R	S	R	R	R	R	S	S
Sc12	R	R	R	S	S	R	S	S
Sc13	R	R	R	S	S	R	S	R
Sc14	R	R	R	R	R	R	S	S
Sc15	R	R	R	S	R	R	S	S
Sc16	R	S	S	R	S	R	S	S
Sc17	R	R	R	S	R	R	S	S
Sc18	R	R	R	S	S	R	R	R
Sc19	R	R	R	S	R	R	R	S
Sc20	R	R	R	R	R	R	R	R

S-Staphylo, c-coccus, a-aureus, M-Methicillin.

V-Vancomycin, K-Kanamycine, A-Ampicillin, T-Tetracycline,

C-Chloromphenicol, E-Erythromycin.

Results and Discussions:-

On the basis of their pigmentation, two types of Staphylococci have been identified. Strain forming yellow coloured colonies a *S. aureus*(*Rosenbach.*) (Gr. Staphylo-cluster of grapes: Gr.coccus-a grain or berry), *S. albus*(*Rosenbach.*) forming white colour colonies, and *S. citreus*(*Rosenbach.*) based on its formation of lemon colour colonies[22].

The present investigations were done with special motive to screen out pathogenic Staphylococci from phylogenic lesion with the internet of Multi-Drug Resistance *Staphylococci aureus* (*Rosenbach.*) (MDRSA). Total 20 Hospital wastes samples were screened out from different clinical sites (viz, post operative wound infections[1]. Many scientist were able to report the synergistic interaction of multiple antibiotics were effective to treat the MRSA infection[14]. Some of the scientist were successful in reporting the *Staphylococcus* cluster forming gram positive cocci, burn infections, and different skin infections [6]. All the cultures were studied for colony characterizations by streaking four ways on Mannitol salt agar and were incubated at 37^0C for 24 hours, and the characters of single well isolated colonies were checked by gram reaction. All isolates were found to be gram positive cocci in bunches. The results obtained from colony characterization, biochemical study, and antimicrobial studies were summarized in table no. 1, table no. 2, and table no. 3 respectively. After performing the morphological and cultural characters, it has been reported that, all the species were gram positive staining, cocci shape, non-motile in tetracycline 30µg and were resistant for species Sca-2 Sca-6, Sca-11, Sca-14, Sca-16 and Ska-20, while, other species were sensitive for tetracycline, motility, convex in elevation, opaque in opacity, and moist in consistency. Few renowned scientist were able to reported the up to 60% nosocomial infections of *Staphylococci aureus*(*Rosenbach.*) were reported resistant to oxacillin[18].But species number Sca-5, Sca-10 was irregular and Sca-18 was regular in margin, while, all others were entire in margin. Species number Sca-1, Sca-3 to Sca-6, Sca-13 to Sca-16 and Sca-20 were pink in colour-Table no.1[20]. After biochemical characters like IMViC, it was evident that, the species were Indole(I) negative, Methyl-red(Mr) positive,Voges-proskauer(VP) positive and Citrate(C) negative(+,+,..) and Carbohydrate(C) test were found that, all species were positive in Glucose, Lactose, and in Raffinose. (Table no. 2). From the results of antibiotic sensitivity tests, it was reported that methicillin 5µg were resistant for all species from Sca-1 to Sca-20. There were reports that, the more than 60% isolates were resistant to methicillin[15]. Vancomycin 30µg were resistant for all species, but sensitive for Sca-11 and Sca-16 similar findings were were also reported[17, 21, 23]. Reports of the glycopeptides antibiotic eg. Vancomycin, an example and currently used for MRSA treatment, clinical isolates of MRSA with resistant to new classes of antibiotics have been reported. Kanamycine 30µg were resistant for all species only species Sca-16 was sensitive. Ampicillin 10µg were resistant for species Sca-1, Sca-4, Sca-11, Sca-15, Sca-17, and Sca-20. While others was sensitive for ampicillin. Similar observations, where the individual application of ampicillin, tetracycline, and amikacin, the MIC range against all strains were of reduced MIC were also reported by many workers[13] . Penicillin 10µg/l were resistant for all species. Similar findings were also reported by i.e. 100% resistance against all antibiotics, they also reported all strains of Pt A, Pt B, Pt C, Pt D and Pt E were shown the 100% resistance[13]. Chloramphenicol 30µg were resistant for species Sca-18, Sca-19, Sca-20, while other species were sensitive erythromycin 10µg were resistant for

species Sca-8, Sca-13, Sca-18, Sca-19 and Sca-20, while others were sensitive for Erythromycin (Table no.-3). Thermal injury (Burn, Surgical wounds, blood from transfusion etc., were reported to be the common sources for *S. aureus*(*Rosenbach.*) for 44%, 34%, 12% respectively. The rate of burn would colonization with *S.aureus*(*Rosenbach.*) varies from 15-85% provided by health care. The major source of microorganism includes the hospital air, cloths, dusts, forming aerosols, hands of contaminated health care workers and colonizing in wound infections. The identification of *Staphylococcus* (*Rosenbach.*) in clinical and hospital waste samples and detection of antimicrobial resistance against various antibiotics in the identified *Staphylococcus*() strains is crucial and important for appropriate microbial therapy and its epidemiology[6]. The isolated strains were screened for different antibiotic resistance by conventional disc diffusion technique. The results obtained by disc diffusion method were of 51 isolates i.e. 85% of *Staphylococci*(*Rosenbach.*) were resistant to methicillin in the present study. Our observations indicates that the epidemiology of MDRSA in our country is also changing over the past few decades it was total 85% of *S. aureus*(*Rosenbach.*) isolates were found to be resistant to methicillin in the present study while in the previous studies, the incidence was found to be 32.8% in 1994, 24% in 1996, 32% in 1997 and 51.6% in 2001[19,24].

Conclusion:

From the results, and analysis, it was recognised that the management of hazardous hospitals wastes is not only a technical problems, but it is intimately influenced by cultural, social and economic circumstances. At the local level hospitals are encouraged to work together to address the economic public health and environmental impact concern of hospital waste management. High level awareness should be created among clinicians and microbiologists about the existence of these pathogens in hospital and infections in coming years. From the Result and Discussion, it was concluded that that the over use of drugs, intravenous drug abuse, incorrect diagnosis, unnecessary prescription improper use of antibiotics by patients and improper use of antibiotics as livestock food additive for growth promotion which leads to mutation and thus leading to mutation and thus leading to drug resistance. For healing the wounds, cuts and scrapes of skin should kept clean and covered with a bandages. Lastly, it is concluded that the researches, pharmaceuticals and scientist must develop such multi disciplinary drugs which can controls such type of organisms or genetic barriers and must develop the proper vaccines or gene therapy, etc to target the control of infections and it is the need of hour too.

Where Sca=S-Staphylo, c-coccus, a-aureus.

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Future Implications: Doctors may thinks about exact prescription while writing medicine. They take the precaution of surgical waste and their management. They may educate the people through the clinical/health camps. Educationist may take the programmes about clinical literacy. Literate people may take the advantage of this study.