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Antibiotics susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* isolated from the clinical samples different tertiary care hospitals of Kathmandu .

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Abstract

The study was carried out in three different tertiary care hospitals of Kathmandu, Nepal. The objective of the study is to isolate and identify bacteria responsible for the different types of infection and to assess antibiotics susceptibility pattern of methicillin resistant Staphylococcus aureus(MRSA) from the clinical samples in the clinical settings. Various clinical samples as wound, pus, bed sore, throat swab, urine and blood were collected from the patients visiting the tertiary care hospitals of Kathamndu. Kirby Bauer disc diffusion test was done to detect antibiotic susceptibility whereas cefoxitin (30mcg) disc was used to detect MRSA. Out of 222 samples, the S. aureus isolated was 103(46.4%).Among the S. aureus, 22(21.3%) were methicillin resistant 98.7% of MSSA were sensitive to Meropenem followed by Amikacin and Tetracycline, 97.5% each respectively. MRSA were sensitive to Chloramphenicol (77.2%) followed by Meropenam (72.7%). This study suggests that very few antibiotics are effective against MRSA and therefore there should be rationale use of antibiotics and antibiotics should be prescribed only after susceptibility test.

Key words: Infection, *Staphylococcus aureus*, Antibiotic Sensitivity.

Introduction

Staphylococcus aureus, although normal flora, is an important human pathogen causing serious and invasive diseases. It has been estimated that *S. aureus* can transiently colonize up to 60% of the human population (CDC, 2013). It causes staphylococcal infections including food poisoning, minor skin abscesses to more serious invasive diseases endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and septicemia(Mukhiya et al., 2013) .*S. aureus* has ability to colonize in the skin and mucous membranes. Hospitalized patients and health-care workers have higher colonization rates than the general population (Asghar, 2014).

The various classes of antibiotics have been found effective against *Staphylococcus aureus*. Antibiotics, such as Vancomycin, Clindamycin , Tetracycline and Fusidic acid are used predominantly for treatment of stubborn staphylococcal infection or in case of allergic reaction from Penicillin group.(Berglund, 2008). Antibiotic resistance has become one of

the challenges in medical science. Among the many species of antibiotic-resistant bacteria, Methicillin-resistant *S.aureus* (MRSA) is one of the most important causes of antibiotic treatment failure, increased morbidity and mortality (Orth et al., 2006). Usually the MRSA strains have become life threatening worldwide due to their resistance to most antibiotics which are abundantly used in clinical practice. A better understanding of the biochemical and genetic mechanisms of *S. aureus* pathogenicity will lead to improved prevention and treatment strategies.(Plata, Rosato, & Wegrzyn, 2009)

MRSA strains are involved in hospital epidemics. The incidence of MRSA infections is increasing worldwide and in some locations exceeds the incidence of Methicillin sensitive *S. aureus*(MSSA) infections (Shrestha, 2013) . Regular monitoring of antibiotic resistance in *S. aureus* isolates in clinical setting is essential for rationale treatment. Further, antibiotic resistance pattern of MRSA is particularly important to holistically design effective treatment of *S. aureus*. Therefore, we conducted this study to assess antibiotic susceptibility pattern of *S. aureus* focusing on MRSA.

Methodology

Study design and Population

The hospital based cross-sectional study was conducted in the first trimester of the year 2020.The study was conducted at three different tertiary care hospitals of Kathmandu valley. Various clinical samples pus, aspirate, throat swab, urine and blood were collected from the patients visiting Sankarapur Hospital, Dirghyau Guru Hospital and Bhaktapur Cancer Hospital. The patients participated for the research were the admitted patients and those hospital visiting as well .

Ethics statement

This study received ethical approval from Nepal Health Research Council (Regd no.114/2020). Suspected patients who agreed to participate and give informed consent were included in the study. Patients who didn't show interest and those who had taken antibiotic less than 48 hours were excluded from the study.

Sample collection and processing

A total of 103 *S. aureus* were isolated from the 222 clinical samples from three different hospitals. Pus and throat samples were collected using sterile cotton swab, patients were asked to collect the midstream urine in the sterile container and for the blood and aspirates, the clinician were requested to take the samples from the patients.

The different clinical samples collected in the hospitals were inoculated in different bacteriological culture media depending upon the type of the specimen. The media used

were MacConkey agar, blood agar and nutrient agar. The specimens were inoculated and incubated at 37ºC for 24 hours (Cheesbrough, 2006). The possible *S. aureus* colonies after Gram staining were inoculated into the selective medium mannitol salt agar. Mannitol salt fermenting yellow colonies were further sub-cultured in nutrient agar and the colonies were subjected to catalase, coagulase, and oxidative and fermentative test.

Antimicrobial susceptibility testing (AST)

S. aureus identified from the morphological and biochemical analysis were further tested against Cefoxitin (30µg) disk using modified Kirby Bauer disk diffusion method using the Clinical and Laboratory Standard Institute (CLSI) guidelines (2014) to detect MRSA isolates. Turbidity of the broth culture was equilibrated to match 0.5 McFarland standards. All bacterial isolates were tested for other antibiotics such as Ampicillin, Cotrimoxazole, Gentamycin, Ciprofloxacin, Cefotaxime, Amikacin, Chloramphenicol, Vancomycin, Meropenem, Tetracycline, Gentamycin and Erythromycin. Nitrofurantoin was used only for the urine samples (CLSI, 2014).

Data analysis

The data were entered into Microsoft Excel and analyzed to get descriptive statistics.

Result and Discussion

The 222 samples were collected from three different three tertiary care hospitals of Kathmandu. Total 222 samples collected as pus, aspirate, throat swab, urine and blood. Out of 222 samples, the *Staphylococcus aureus* isolated were 103(46.4%).Among the *Staphylococcus aureus*, 81 (78.65%) isolated as Methicilin Sensitive *Staphylococcus aureus* and 22(21.3%) as the Methicillin Resistant *Staphylococcus aureus*.Out of three hospitals, the maximum number of MRSA were isolated from Bhaktpapur Cancer Hospital (16.5%) followed Dirghayu Guru Hospital (2.9%) and Sankarapur Hospital (1.9%) (Table1).

Table 1

SN	Hospital	Specimen no	*S,aureus* isolates	Clinical sample					MRSA	%	MSSA	%
				Pus	Aspirate	Urine	Throat Swab	Blood				
1	SPH	51	**21**	18	0	0	2	1	**2**	1.9	**19**	18.44
2	DGH	103	**53**	43	10	0	0	0	**3**	2.9	**50**	48.5
3	BCH	68	**29**	10	0	13	4	2	**17**	16.5	**12**	11.65
Total		**222**	**103**	**71**	**10**	**13**	**6**	**3**	**22**	21.3	**81**	78.6

Antibiotic susceptibility pattern of MSSA showed 98.7% of MSSA were sensitive to Meropenem followed by Amikacin and Tetracycline, each having 97.5%. MRSA were

resistant to most of the antibiotics.It was found sensitive against Chloramphenicol (77.2%) followed by Meropenem (72.7%).MRSA were found resistant against Ampicillin (95.4%) followed by Cefotaxime (90.9%) (Table 2).

Table 2

AST of MSSA					
Antibiotic	**SEN**	**%**	**RES+I**	**%**	**Tot**
Ampicillin (AMP)	36	44.44	45	55.5	81
Cotrimoxazol(COT)	65	80.24	16	19.7	81
Cefotaxim(Ctx)	53	65.4	28	34.5	81
Gentamycin(GEN)	75	92.6	6	7.4	81
Amikacin(AK)	79	97.5	2	2.4	81
Chloremphenicol©	70	86.4	11	13.5	81
Erythromycin(Ery)	69	85.1	12	14.8	81
Meropenam(Mero)	80	98.7	1	1.2	81
Vancomycin(Van)	73	90.1	2+6	12.2	81
Tetracycline(Tet)	79	97.5	2	2.4	81
Ciprofloxacin(Cip)	54	66.6	27	33.33	81

According to Table 3, MRSA has been found more resistant to most of the Antibiotic .It was found sensitive against Chremphenicol 77.2% followed by Meropenam 72.7%.MRSA found most resistant against Ampicillin 95.4% followed by Cefotaxim 90.9% .

Table 3

AST of MRSA					
Antibiotic	**Sen**	**%**	**Res**	**%**	**Total**
Ampicillin(AMP)	1	4.5	21	95.4	22
Cotrimoxazol(COT)	11	50	11	50	22
Cefotaxim(Ctx)	2	9.1	20	90.9	22
Gentamycin(GEN)	8	36.3	14	63.6	22
Amikacin(Ak)	15	68.1	7	31.8	22
Chloramphenicol©	17	77.2	5	22.7	22
Erythromycin(Ery)	4	18.2	18	81.8	22
Meropenem(Mero)	16	72.7	6	27.3	22
Vancomycin(VAN)	7	31.8	15	68.1	22
Tetracycline(Tet)	13	59	9	40.9	22
Ciprofloxacin(CIP)	6	27.3	16	72.7	22

Discussion

The study determined the prevalence of *S. aureus* infections in a tertiary care hospitals. Majority were isolated from pus followed by urine and the least from blood.

The significantly higher rate of MRSA infection observed in Bhaktapur Cancer Hospital as compared to other two hospitals can be justified by the fact that the cancer hospital may have the patients with the multiple use of antibiotics and may be the higher numbers of referral cases. The extent of MRSA infection can be justified by the fact that once MRSA becomes endemic within a hospital setting, such resided bacteria may report for 5-50% of all nosocomial *S. aureus* infections in that hospital (Shrestha, 2013). Infection control program should be effectively implemented to control the transmission of MRSA among the patients in the hospital.

Our study suggested that MSSA were sensitive to Meropenem (98.7%) followed by Amikacin and Tetracycline. The MSSA also showed sensitivity to other antibiotics as well and the resistance against Cotrimoxazole was 34.5%. A study in Nepal also reported similar results of Cotrimoxazole which provides evidence that it is least effective antibiotic for Gram positive isolates(Rijal, Satyal, & Parajuli, 2017).

The study showed that all the MRSA isolates were resistant towards the most of the commonly used antibiotics compared to that of MSSA.Antibiotic susceptibility test showed that in case of the MSSA strain, most of them showed sensitivity against most of the antibiotics, except the widely used Ampicillin.The resistance against Ampicillin might be due the non-compliance of the antibiotic, irrational use, and self-medication practices. The study carried out in Nepal showed that among the MSSA isolates, most were susceptible to many of the tested FDA recommended antibiotics(Shrestha, 2013).

Conclusion

MRSA strains are challenge to the clinical sector as it is being resistant to a wide range of antibiotics. The formulation of a definite antimicrobial policy may be helpful for reducing MRSA prevalence in hospital setting. This study also concludes that very few antibiotics are effective against MRSA and therefore there should be rationale use of antibiotics and antibiotics should be prescribed only after susceptibility test. Regular monitoring of antimicrobial susceptibility pattern of MSSA and MRSA may reduce the nosocomial infection. In addition, continuous surveillance of microbial flora , adopting the standards hygienic behavior in hospitals among the health care providers and the health care seekers will help to prevent from *Staphylococcus aureus* transmission .

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