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Original Research Article

PHENOTYPIC CHARACTERIZATION AND ANTIBIOGRAM OF STAPHYLOCOCCAL ISOLATES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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Abstract

Background: Staphylococci, actually part of the normal human microbiome have also got the propensity to cause a diverse array of life threatening infections. It has overcome most of the therapeutic agents developed over the recent years and many of them are becoming multidrug resistant as well. Coagulase negative staphylococci are also gaining significance now especially in critically ill patients leading to increased morbidity and mortality. The objective of this study is to characterize staphylococci obtained from various clinical samples, to find its antibiogram with special reference to its resistance pattern so that stringent measures can be applied to control their spread. **Materials and Methods:** Study sample was selected from the Staphylococcal isolates obtained from routine clinical samples during a period of 1 ½ years in the Department by systematic sampling method. The isolates were phenotypically characterized and their antimicrobial susceptibility was tested and interpreted as per the CLSI guidelines. The isolates were also screened for Methicillin and Vancomycin resistance and their susceptibility to newer antistaphylococcal agents were also tested. **Result:** Out of the 300 Staphylococcal isolates included in the study, 90.7% were coagulase positive and 9.3% coagulase negative. It consisted of 34.7% MRSA, 56% MSSA, 5.7% MRCONS and 3.6% MSCONS. Resistance to Penicillin and Ciprofloxacin was exhibited by 90% and 70.6% of S aureus respectively. All the staphylococcal isolates were found to be sensitive to Vancomycin, Linezolid, Daptomycin and Teicoplanin. Resistance to Rifampicin was exhibited by 7 staphylococcal isolates (6 MRSA and 1 MSCONS). **Conclusion:** Staphylococci are still an important hospital and community acquired pathogen. There should be regular surveillance for multidrug resistant strains which will be useful for selection of appropriate antibiotic, to know the changing trends of antibiotic susceptibility pattern and also for limiting the use of higher antibiotics, which can be conserved for treating resistant and life-threatening staphylococcal infections.

INTRODUCTION

Staphylococcus, a frequent colonizer of the human respiratory tract is also notorious for causing a diverse array of life threatening infections.[1] Cell associated polymers, cell surface proteins, extracellular enzymes and toxins contribute to its virulence. The emergence of antibiotic resistant Staphylococcus aureus (eg.MRSA) is a worldwide problem in clinical medicine. By 1950, 40% of hospital Staphylococcus aureus (S aureus) isolates were penicillin-resistant; and, by 1960, this had risen to 80%.[2] Methicilin resistant strains, which were most often found associated with hospitals are becoming increasingly prevalent in community acquired infections as well. Quinolone as well as Clindamycin resistance among S.aureus emerged quickly, more prominently among MRSA, dramatically reducing its use as an antistaphylococcal agent.[1]

Vancomycin-resistant S.aureus(VRSA) is a strain that has become resistant to glycopeptides. The first case of Vancomycin-intermediate S.aureus (VISA) and VRSA were reported in 1996(Japan) and 2002 respectively.[3,4]

Coagulase-negative Staphylococci (CONS) have become recognized as an important pathogen in immunocompromised patients and its clinical

significance is increasing with introduction of more invasive procedures.[5] Since they are widespread on the human body and can form large populations, distinguishing the etiologic agent(s) from contaminating flora is a serious challenge. Clinical correlation and repeated isolation are needed to ascertain their clinical significance.

One of the difficult therapeutic problems of multidrug-resistant S.aureus is the diminishing efficacy of antimicrobial agents for the treatment of bacterial infections.[6] This trend is particularly alarming for Staphylococci because of the severity and diversity of disease caused by this uniquely versatile pathogen. While effective anti-staphylococcal agents still exist, their shelf-life is likely to be increasingly limited. Novel approaches to therapy and prevention will become more and more important, especially with the diminishing availability of new "wonder drugs."

So this study which was carried out for a period of 1 ½ years in Jubilee Mission Medical College and Research Institute, Thrissur, Kerala, aims to find out the antibiotic sensitivity pattern of Staphylococci isolated with special reference to its resistance pattern and to apply stringent measures to control the spread.

MATERIALS AND METHODS

The study was conducted in the Department after getting approval from the Institutional Ethics committee. It was carried out on isolates of *Staphylococcus* obtained from various clinical samples during one and half year (18 months) period received in the microbiology laboratory. The various samples from which the isolates were obtained include pus, blood, urine, CSF, respiratory samples, umbilical vein catheter and central venous catheter. The samples which were collected in appropriate containers by the treating doctors were received and processed in the routine microbiology laboratory. On reaching the laboratory, all the clinical specimens were first inoculated onto Blood agar and MacConkey agar plates (Hi Media Mumbai India) and these were incubated at 370C for 24-48 hours as per the standard microbiological methods. Colony characteristics were examined and Gram staining of the isolates was done. Isolate that shows Gram positive cocci in groups were further processed and identification done by standard biochemical tests. All Gram positive cocci in clusters that are Catalase positive were divided into 2 groups as Coagulase positive Staphylococci *(S. aureus)* and Coagulase negative Staphylococci (CONS) by Slide coagulase and Tube coagulase test [Figure1, 2].

As per the suggestions from the statistician based on the previous prevalence data from our centre, a minimum of 300 clinically significant Staphylococcal isolates has to be included in this study. In case of CONS, pure growth with plenty of pus cells as well as repeated isolation is warranted. Staphylococcal isolates from environmental samples were not included.

All confirmed *Staphylococcus* strains were subjected to routine Antimicrobial susceptibility testing (AST) using manual and automated methods [Vitek 2 compact systems (bioMerieux Ltd, using GP67 cards)]. The antibiotics used for AST were Penicillin (10U), Cephalexin (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Oxacillin (1 µg) and Cefoxitin (30 µg). Quality control tests were carried out with the Standard ATCC strains of *Staphylococcus aureus 25923*. Along with routine AST, all the isolates were screened for Methicillin resistance and Vancomycin resistance as per the CLSI guidelines[7]. Methicillin resistance was detected using Oxacillin disc diffusion test[7,8], Cefoxitin disc screen test[7,8] and Oxacillin Screen Agar test[7,8] [Figure 3]. Detection of *Vancomycin Intermediate S aureus* (VISA) / *Vancomycin Resistant S aureus* (VRSA) was done using Vancomycin Agar Screen test[7,8]. Susceptibility to newer anti-staphylococcal agents like Linezolid, Daptomycin, Rifampicin and Teicoplanin were tested using Vitek 2 Compact systems (Biomerieux Ltd, using GP67 cards). All the instructions of the manufacturers were strictly followed during processing.

Statistical Analysis

Epi info (version 7.1.4.0) Centre for Disease Control and prevention (CDC), Atlanta, Georgia was used for analysis and interpretation of the results. p value was calculated to find out statistical significance. The results were reported as percentages. A p value of ≤ 0.5 is considered as significant association.

RESULTS

The initial 1 ½ years was used for sample collection and processing and the remaining 3 months were taken for data generation. A total of 1868 samples were received in the microbiology laboratory for routine bacterial culture during the study period which includes 386 MRSA, 667 MSSA and 815 CONS. Isolates were included in the study based on their clinical significance. The samples were processed as per the standard microbiological methods.

A total of 300 non-duplicate Staphylococcal isolates were included in the present study. All the confirmed Staphylococcal strains were subsequently tested for antibiotic sensitivity on Mueller Hinton Agar (MHA) using commercial antibiotic discs (HiMedia) by standard Kirby-Bauer disk diffusion method and interpreted as per the CLSI recommendations and also by the Automated method using Vitek 2 compact systems (bioMerieux Ltd, using GP67 cards). The isolates were screened for Methicillin resistance and Vancomycin resistance as per the CLSI guidelines.

Samples from different clinical departments were analysed. The data shows that maximum number of isolates was obtained from the department of surgery (125), out of which 120 were from pus samples. This is followed by the ICU/CCU (49), Medicine (42) and orthopedics (30). Sample wise analysis was also

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performed. About 79.3% of the staphylococcal isolates were from pus, 9% from respiratory secretions, 7.6% from blood samples, 3% from urine and 0.33% from CSF, CVP and UVC each [Figure 4]. Out of the 300 isolates 104 were MRSA (34.7%), 168 MSSA (56%), 17 MRCONS (5.7%) and 11 MSCONS (3.6%) [Table: 1]. Among the 28 CONS isolated, Staphylococcus haemolyticus was the commonest species [8 (28.6%)], followed by S epidermidis [5 (17.9%)], S saprophyticus [3 (10.7%)] and S lugdunensis [1 (3.6%)]. Around 11 isolates of CONS could not be speciated in the present study.

Antibiotic susceptibility of methicillin sensitive strains was compared with that of methicillin resistant staphylococcal isolates [Table: 2]. Among S aureus, only 16.07% of MSSA were found to be sensitive to penicillin. Among MSSA, only 4.80% and 0.60% of the isolates was resistant to Gentamicin and Amikacin respectively whereas in case of MRSA, 43.27% and 16.35% were resistant to the same antibiotics respectively. The percentage of resistance to Ciprofloxacin in case of MRSA and MSSA isolates were 87.50% and 60.12% respectively. Co-existing resistance to different antibiotics with methicillin resistant strains was significantly higher in comparison to methicillin sensitive strains (based on the p values), exception being Vancomycin which gave a higher value.

Among CONS, only 18.18% of MSCONS were found to be sensitive to Penicillin. All MSCONS were sensitive to Amikacin, whereas 5.90% of MRCONS exhibited resistance. Resistance to Ciprofloxacin was exhibited by 64.70% of MRCONS and 36.36% of MSCONS [Figure 5]. But when the antibiogram of MRCONS and MSCONS was compared statistically, no significance was found. All the isolates were screened for Vancomycin resistance using the Vancomycin agar screen test and none of them were found to be resistant to the drug.

The susceptibility of the newer anti-staphylococcal agents was tested by using the automated method, Vitek 2 compact systems (bioMerieux). Linezolid, Daptomycin and Teicoplanin were found to be sensitive in all of the 236 isolates tested. Resistance to Rifampicin was shown by 7 isolates (3%), which consists of 6 MRSA isolates and 1 MSCONS [Table 3].

Figure 1: Colonies of Staphylococcus aureus in Blood agar

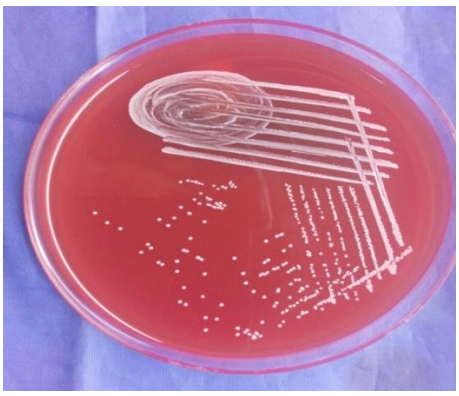

Figure 2: Colonies of Coagulase negative staphylococci in Blood agar

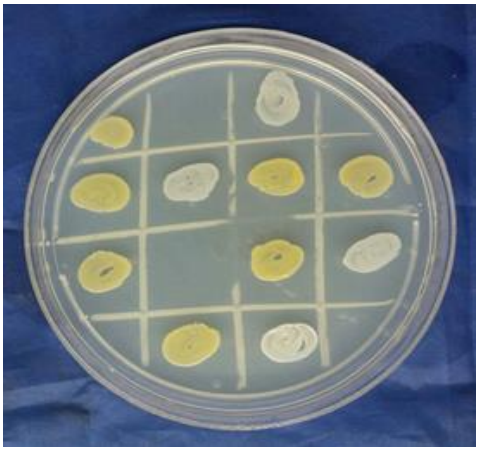

Figure 3: Oxacillin Screen agar test

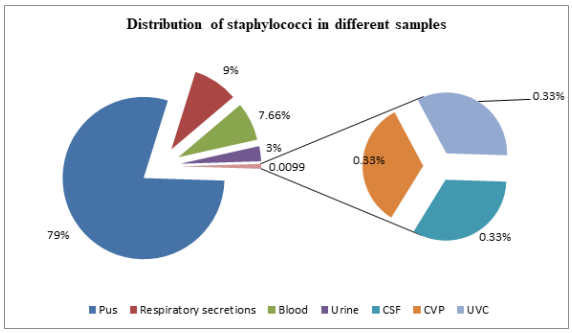

Figure 4: Distribution of staphylococci in different samples (n = 300)

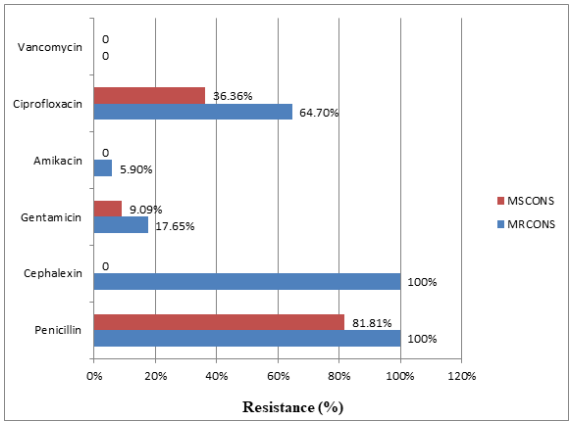

Figure 5: Antibiotic susceptibility profile of MRCONS and MSCONS

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Table 1: Distribution of isolates based on Coagulase test (n=300).

	Coagulase Positive		Coagulase Negative		Total
	MRSA	MSSA	MRCONS	MSCONS	
Pus	80	142	9	7	238
Blood	7	12	3	1	23
Urine	2	5	-	2	9
Respiratory sample	13	9	4	1	27
CSF	-	-	1	-	1
CVP	1	-	-	-	1
UVC	1	-	-	-	1
Total	104	168	17	11	300

Table 2: Antibiotic susceptibility pattern of MRSA and MSSA

Antibiotic	Sensitive		Resistant		p value
	MRSA	MSSA	MRSA	MSSA	
Penicillin	0	27 (16.07%)	104 (100%)	141 (83.93%)	0.0001
Cephalexin	0	168 (100%)	104 (100%)	0	0.0001
Gentamicin	59 (56.73%)	160 (95.23%)	45 (43.27%)	8 (4.80%)	0.0001
Amikacin	87 (83.65%)	167 (99.4%)	17 (16.35%)	1 (0.60%)	0.0001
Ciprofloxacin	13 (12.5%)	67 (39.88%)	91 (87.50%)	101 (60.12%)	0.0001
Vancomycin	104 (100%)	168 (100%)	0	0	1.0000

Table 3: Antibiotic susceptibility to newer anti-staphylococcal agents

No	Antibacterial agent	Sensitive		Resistant	
		S aureus	CONS	S aureus	CONS
1	Linezolid	100%	100%	0	0
2	Daptomycin	100%	100%	0	0
3	Teicoplanin	100%	100%	0	0
4	Rifampicin	97.8%	96.43%	2.20%	3.57%

Table 4: Percentage of *S aureus* in various samples

Study conducted by	Pus	Respiratory secretions	Blood	Urine	CSF	UVC & CVP
Rajaduraipandi K et al[9] (2006)	50.7%	6.4%	9.7%	1.6%	-	-
ShanthiM et al[12] (2009)	80%	2.5%	12.5%	5%	-	-
Kulkarni S et al[22] (2014)	42.6%	6%	10%	17.26%	-	-
Mantri SR et al[13](2014)	74.1%	19.64%	3.57%	4.46%	-	-
Shahi K et al[14] (2018)	70.6%	7.3%	-	11.9%	-	-
Pradhan P et al[15](2021)	78.6%	4.1%	7%	4.1%	-	-
Our study	81.6%	8.09%	6.9%	2.6%	0	0.7%

Table 5: Distribution of *Staphylococci* in various studies

Study conducted by	Organism			
	MRSA (%)	MSSA (%)	MRCONS (%)	MSCONS (%)
Angel MR et al[20] (2008)	24.57	53.81	21.61	
Shanthi M et al[12] (2009)	45	55	-	-
Joshi S et al[19] (2013)	42	58	-	-
Mantri SR et al[13](2014)	39.29	28.07	4.21	28.42
Jayanthi RS et al[16] (2015)	-	-	11.22	22.45
Pradhan P et al[15] (2021)	40	60	-	-
Alam F M et al[21] (2022)	41.1	58.9		
Our study	34.7	56	5.7	3.6

Table 6: Percentage of resistance to antimicrobials by MRSA strains

Drug	Our study	Kulkarni S et al[17] (2014)	Mantri SR et al[12](2014)	Joshi S et al[15] (2013)
Penicillin	100%	-	-	100%
Cephalexin	100%	-	-	-
Gentamicin	43.27%	52.57%	68.75%	58.3%
Amikacin	16.35%	44.13%	-	-
Ciprofloxacin	87.50%	74.06%	22.32%	79.3%
Vancomycin	0	0	0	0

DISCUSSION

Staphylococci are a group of bacteria that are commonly found in humans and animals. They can either be part of the normal flora or can be pathogenic depending on the strain and the health status of the person. The most pathogenic species is Staphylococcus aureus which can cause a diverse array of infections ranging from minor skin infections to life threatening diseases like pneumonia and septicemia.

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It has overcome most of the therapeutic agents that have been developed over the recent years, most notable example being the emergence of MRSA, which was reported just one year after the launch of methicillin. Many of these isolates are becoming multidrug resistant also and becoming susceptible only to higher antibiotics like vancomycin. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness etc are the possible predisposing factors for the emergence of MRSA as well as other multidrug resistant staphylococcal strains.[9]

Coagulase negative staphylococci were generally regarded as contaminants, having little clinical significance in the past. But now it is being recognized as a major cause of nosocomial infections especially in critically ill patients leading to significant morbidity and even mortality.[10]

The development of multidrug resistance and the control of spread of these isolates in hospitals as well as in the community have been recognized as the major challenges as the bacterial population that expresses this resistance phenotype varies according to the environmental conditions.[11] Therefore the knowledge about the different phenotypes prevalent in the area as well as their current antibiogram becomes necessary in the selection of appropriate empirical treatment of these infections. In the present study we tried to find out the antibiogram of the staphylococci isolated with special reference to its resistance pattern so that stringent measures can be applied to control their spread.

A total of 300 clinically relevant non-duplicate staphylococcal isolates were enrolled in the present study conducted over a period of 1 ½ years in our department. Isolates were analyzed based on the department from where they arrived as well as by the sample type.In the present study, 42% of the isolates were from the department of surgery. This is natural since staphylococci are one of the main agents causing suppurative lesions. This was followed by the ICU/CCU (16.33%), Medicine (14%) and orthopedics (10%).

Of the 300 isolates, 272 (90.7%) were Staphylococcus aureus and 28 (9.3%) were coagulase negative staphylococci. Out of the 272 S aureus strains, 81.6% of the clinical samples were from pus, 8.09% from respiratory secretions, 6.9% from blood, 2.6% from urine and 0.7% from UVC and CVP together. This was almost in accordance with the study conducted by Rajaduraipandi K et al,[9] exception being the proportion of pus samples (50.7%) which is lower when compared to our study. The proportion of pus samples in our study was similar to the studies conducted by Shanthi M et al (80%), Mantri SR et al (74.1%), Shahi K et al (70.6%) and Pradhan P et al (78.6%) [Table: 4].[12-15]

Among the 28 CONS isolated in our study, 16 (57.14%) isolates were from pus samples, 5 (17.9%) from respiratory secretions, 4 (14.3%) from blood, 2 (7.14%) from urine and 1 (3.6%) from CSF.In the study conducted by Jayanthi RS et al,[16] as in our study, pus samples contributed to about 54.54%, but others are different as, blood 24.24%, urine 18.2% and CVP 3%. Meanwhile blood samples predominated over pus samples in the study conducted by Usha MG et al,[10] (53% and 32.35% respectively).

The age groups that were seen to be highly infected with staphylococci were 41-60 years (53.3%) followed by > 60 years (24.7%) and 21-40 years (20.3%). Male were 193 (64%) and females were 107 (36%). In the study by Kali A et al,[17] a total of 75 patients (73.52%) were in the 16-60 years age group. As per ICMR 2023 report,[18] the overall proportion of MRSA reported from different centres across India was 44.5% - 64.1% and resistance in CONS was upto 80%. In our study, MSSA predominated over MRSA, MRCONS and MSCONS (56%, 34.7%, 5.7% and 3.6% respectively).These results are found to be almost in accordance with Shanthi M et al,[12] (55% MSSA), Joshi S et al (58% MSSA),[19] Angel MR et al,[20] (53.81% MSSA) and Alam F M et al (58.9% MSSA).[21] In contrast to this, MRSA was found to predominate over MSSA in the study conducted by Mantri SR et al 13 (39.29% MRSA, 28.07% MSSA) [Table: 5].

In the study conducted by Angel MR et al[20] the prevalence of CONS was 21.61%. The prevalence of MRCONS and MSCONS in the studies conducted by Mantri SR et al,[13] is 4.21% and 28.42% and that of Jayanthi RS et al[16] is 11.22% and 22.45%.

Among the 28 CONS isolated, Staphylococcus haemolyticus was the commonest species [8 (28.6%)], followed by S epidermidis [5 (17.9%)], S saprophyticus [3 (10.7%)] and S lugdunensis [1 (3.6%)]. This was in accordance with the ICMR 2023 report.[18]

In the present study MRSA strains showed the highest rate of resistance to Ciprofloxacin (87.5%). This was in accordance with the study conducted by Joshi S et al and Kulkarni S et al.[19,22] Contrary to the reports by Mantri SR et al,[13] who reported 22.32% resistance to Ciprofloxacin that is much lower when compared to our study [Table: 6]. Among MSSA, 83.93% exhibited resistance to Penicillin followed by 60.12% to Ciprofloxacin. Resistance to Amikacin and Gentamicin were very low (0.60% and 4.80% respectively).

Among MRCONS, maximum resistance was shown by Penicillin (100%) and Cephalexin (100%) followed by Ciprofloxacin (64.7%), Gentamicin (17.65%), and Amikacin (5.9%). MSCONS also followed similar pattern with no resistance to Cephalexin and Amikacin. Both MRCONS as well as MSCONS were fully susceptible to Vancomycin, Linezolid and to other newer anti-staphylococcal agents like Daptomycin and Teicoplanin. One MSCONS was found to be resistant to Rifampicin whereas all the MRCONS were fully sensitive to the drug. This was almost in accordance with the study conducted by Jayanthi RS et al,[16] the main

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difference from it being Ciprofloxacin fully sensitive in their study.

Though CONS, which are part of the normal flora of the skin and mucous membrane, are considered less virulent than S aureus, they are the most common cause of prosthetic device infections. As a result of exposure to multiple antibiotics, surgical prophylaxis and indiscriminate use of antibiotics, patients become colonized with multi-drug resistant strains of CONS which has led to the use of glycopeptides in high risk patients.[23] Hence there is a need for accurate identification of these isolates and their antibiotic sensitivity pattern to avoid decreased susceptibility to glycopeptides. Differentiation between culture contamination and true infection is another problem encountered when CONS is grown in culture and so repeated isolation with pure growth as well as presence of plenty of pus cells in microscopy is warranted.

In our study, resistance exhibited by MRSA was found to be higher than that of MSSA and was proved to be statistically significant. But in the case of CONS, we could not yield any statistical significance, most probably due to the low number of isolates of CONS incorporated in the study. All isolates were found to be sensitive to Vancomycin, Linezolid, Daptomycin and Teicoplanin, whereas 7 isolates (6 MRSA and 1 MSCONS) were found to be resistant to Rifampicin.

Despite the recent introduction of antimicrobial agents and medical improvements in controlling the frequency and morbidity of staphylococcal infections, they are still persistent as an important hospital and community acquired pathogen. Most common reason for multi-drug resistant MRSA is indiscriminate use of antibiotics without drug sensitivity testing which may be due to lack of advanced laboratory facilities or negligence on the part of medical practitioners or poor economic status of the patient. There is a difference between antibiotic sensitivity profiles of MRSA and MSSA isolates and hence routine testing of methicillin resistance should be done using cefoxitin disc which at present is the most sensitive method.[21]

There should be regular surveillance of MRSA and MRCONS which will also be useful for selecting an appropriate antibiotic, to know the changing trends of antibiotic susceptibility pattern, for developing hospital antibiotic policy and also for limiting the use of powerful antibiotics like Vancomycin as initial treatment, so that we can save it for the treatment of resistant and life-threatening staphylococcal infections.[24]

CONCLUSION

Phenotypic characterization and antibiogram profiling of staphylococcal isolates are important since it helps in identification, treatment and control of staphylococcal infections especially those caused by multidrug resistant strains like MRSA. Strict hygienic practices, efficient antibiotic stewardship programme, active screening and compliance with recommended infection control practices play an important role in the control and spread of such multi-drug resistant strains.

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