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A Study on Methicillin Resistant *Staphylococcus aureus* Phage Types in Wound Infections and its Antibiotic Susceptibility Pattern at King George Hospital, Visakhapatnam, India

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A B S T R A C T

Methicillin resistant *Staphylococcus aureus* (MRSA) is a growing concern in the community and in hospitals ever since it has been diagnosed. As staphylococcus has been the dominant pathogen of wounds, Methicillin resistance made the treatment a tough challenge to the clinician. The growing problem of antibiotic resistance and the need for alternative treatments have led to a reappearance of importance in phage therapy. Scope: The present study aimed on MRSA phage types and its antibiotic susceptibility pattern in wounds. Material and Methods: In this study a total of 92 coagulase positive *Staphylococcus aureus* obtained from various wounds are identified by inoculating on Blood agar, MacConkey agar and Nutrient agar and processed further by necessary biochemical tests and tube coagulase tests. All strains were tested for Methicillin resistance and phage typing. Findings: The results obtained were MRSA (22) of 23.92%, Methicillin sensitive staphylococcus aureus (MSSA) (70) of 76.08% and the predominant phage type in MRSA and MSSA obtained was phage III. The typable MRSA strains and MSSA strains showed 100% sensitivity to Vancomycin followed by 89.13% sensitivity to Cefaperazone-Sulbactum, 87.5% to Ciprofloxacin. Conclusion: Phage typing may be a very useful diagnostic method in future.

Introduction

Staphylococcus aureus is one of the most challenging human pathogen causing a variety of infections ranging from relatively mild involvement of skin and soft tissue to life threatening sepsis, pneumonia and toxic shock syndrome. Infections are common in both community and hospital settings

(Fey *et al.*, 2003). The pathogen has a differential capability to spread and cause outbreaks in the hospital as well as in the community. It is very important to characterize staphylococcal strains in order to prevent its spread in hospitals and in the community (Mehndiratta *et al.*, 2010). Methicillin resistant *Staphylococcus aureus* (MRSA) was detected in early 1960's in United Kingdom and has become the major hospital acquired pathogen all over the world is a Multidrug resistant organism which is resistant nearly to every antibiotics with the exception of Vancomycin. It has been observing that community acquired MRSA infections increasing from the past two decades. MRSA has been declared as international concern by the World Health Organisation (WHO) (Masoumeh Hallajzadeh *et al*, 2019). Staphylococcal strains showing Methicillin drug resistance are also raising and spread in the community more rapidly than normal. MRSA is widely spreading the resistant organism of wounds and posing threat in treatment perspective. MRSA shows higher rates of antibiotic resistance to betalactam antibiotics and also to the other antibiotics when compared to Methicillin sensitive *Staphylococcus aureus* (MSSA). The infections caused by MRSA are very expensive to treat even though the virulence is less when compared to MSSA. This is because most of the strains of MRSA show decreased susceptibility to the definitive therapy drugs such as Vancomycin and Teicoplanin. Even though application of strict measures such as reducing hospital stay, drugs susceptibility testing and strict aseptic precautions while handling wound failed in controlling MRSA so it has become necessary to conduct the epidemiological screening of strains by various phenotyping methods such as antibiogram, phage typing, serotyping (Weller, 2000). Discovering completely novel treatment may not be vital instead studying strain specific bacteriophage treatment might be a solution for antibiotic resistance. A further research is essential to validate the efficacy and safety of these treatments before these can be incorporated into standard clinical care (Nathan B. Pincus *et al.*, 2015). In this study the researcher considered two typing methods to screen *Staphylococcus aureus* strains such as antibiogram and phage typing. It has become necessary to evaluate the phage types of staphylococcus as a part of prevention of its spread in the hospitals. Phage typing methods are simple and cost effective and its application needs careful assessment and individual approach for reproducibility of the results (Mehndiratta and Bhalla, 2012).

The present study was conducted to know the existing phage types of *Staphylococcus aureus* in wounds in relation to their Methicillin resistance.

Materials and Methods

This study was done by taking one hundred pus samples from various types of wounds from the inpatient as well as outpatient departments of King George Hospital, Visakhapatnam, Andhra Pradesh, India over a period of one year from 2018 to 2019 after attained approval from Institutional ethical committee.. The samples collected were Gram stained and processed on conventional cultures media such as Blood agar, MacConkey agar and Nutrient agar along with necessary biochemical reactions and finally subjected to antibiogram. *Staphylococcus aureus* strains were confirmed by standard culture techniques based on colony morphology, Gram staining, catalase test, slide coagulase test and tube coagulase test. The MRSA were isolated by modified Kirby-Bauer's method by using Methicillin 5ug disc according to CLSI 2020 guidelines (Clinical and Laboratory Standard Institute). Phage typing was performed at Staphylococcal phage typing center, Moulana Azad Medical College, New Delhi. Phage typing was done by standard methods at I RTD and 100 RTD (routine test dilution) using an international set of 23 phages. The coagulase positive MRSA strains obtained were phage typed by Group I, Group II, Group III and Group IV phages in Moulana Azad Medical College, New Delhi. The Group I phages tested are 29,52C,52A,79,80 followed by Group II 3A, 3C, 55, 71; Group III 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; Group IV 81, 94, 95, 96. All the MRSA and MSSA strains were subjected to

antibiotic sensitivity testing. Antibiotic sensitivity testing was performed using modified Kirby-Bauers method of disc diffusion test. Antibiotic discs used were Methicillin (5µg), Penicillin (10 I.U), Cephalexin (30µg), Gentamicin (10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Clindamycin (2µg), and Vancomycin (30µg).

The strains were inoculated in the separate plates on Mueller Hinton agar for Methicillin testing and these plates were incubated at 37 ^{0} C for 24 hours.

The conformation of all the Methicillin sensitivity was done by oxacillin screening using 6µg/ ml oxacillin in Mueller Hinton agar with 4% Na Cl (Mehndiratta *et al.,* 2010).

Statistical Analysis

To compare the antimicrobial susceptibility between MSSA) and MRSA strains and between phage groups the Author has used Chi square test.

Results: A total 100 pus samples from wounds were processed and obtained 92% culture positivity and all isolates were identified as *Staphylococcus aureus*. Out of 92 *Staphylococcus aureus* 70 (76.08%) were MSSA and 22 (23.92%) were MRSA (Table: 1). All *Staphylococcus aureus* were sent for phage typing at Moulana Azad Medical College, New Delhi.

The typable strains in MRSA were 9 (47.4%) and MSSA were 19 (67.9%). The total number of typable strains was 28 in which the percentage of MSSA and MRSA are 19 (67.85%) and 9 (32.14%), respectively.

Phage group distribution among MSSA and MRSA strains is found as Group III 14 (MSSA) and 8 (MRSA); Group IV 2(MSSA) and 0 (MRSA) and mixed Groups I & III 1 (MSSA) and 1 (MRSA), Group II & III 2 (MSSA) and 0 (MRSA). No strains were found in Group I & Group III. A total number of 92 *Staphylococcus aureus* were phage typed and 64 were non typable (69.56%) 28 were typable (30.44%). Out of the 28 strains that could be typed, 22 strains (78.57%) belonged to group III, two strains (7.14%) belonged to group IV and 4 strains (14.29%) belonged to mixed group (Table: 2).

Antibiogram of the isolates was studied by modified Kirby –Bauer disc diffusion technique and found that all staphylococcal strains were resistant to pencillin due to production of β - lactamase production. *Staphylococcus aureus* showed 100% sensitivity to Vancomycin, Teicoplanin and 100% resistance to Penicillin followed by 50% to Methicillin (Suguneswari *et al.,* 2013).

Results and Discussion

Staphylococcus aureus has been the consistent and predominant organism infecting wounds since long time. Methicillin resistance among the strains posing threat and complicates treatment. It becomes necessary to be aware of the epidemiological relatedness of the strains for successful implementation of treatment methods.

One of the best methods to evaluate different strains among the staphylococcus isolates has been the bacteriophage typing and also has been considered as the best tool to study the relatedness of *Staphylococcus aureus*.

In the present study Methicillin resistance among *Staphylococcus aureus* were 23.92% and MSSA were 76.08% and the 64 were non typable (69.56%) 28 were typable (30.44%). Most typable strains belonged to phage group III which coincides with previous studies Mehndiratta *et al.,* (2010); Asmita *et al.,* (2011) and Mehndiratta and Bhalla (2012).

The predominant phage group in the study belonged to Group III and majority of the MRSA and MRSA were sensitive to this group which coincides with the study of Vazhavandal and Uma (2017) in contrast to Nagwa Mohamed Amin Aref (2014) where the group II phages type was predominated and mixed group predominance followed by Group III in Al-Khulaifi Manal *et al.,* (2009).

Table.1 Methicillin resistant *Staphylococcus aureus*

Staphylococcus aureus	**No of isolates**	**Percentage**
Methicillin sensitive	70	76.08
Methicillin resistant	22	23.92
Total	92	100

Table.2 Phage Pattern of Staphylococcus from cases

Group	Type	No of cases		Group total
		At 1 RTD	At 100 RTD	
I	29 52C 52A 79 80	0	0	0
II	3A 3C 55 71	0	0	0
III	6 42E 47 53 54 75 77 83A 84 85	20	2	22
IV	81 94 95 96	0	2	2
Mixed group	Group I &Group III	2	0	2
	Group II &Group III	0	2	2
Total typable				28
Non typable				64
Total				92

(RTD-Routine test dilution)

Table.3 Various phage groups of *Staphylococcus aureus* and its antibiotic susceptibility patterns.

Staphylococcus aureus	P	M	CP	CfS	V	CD	E	TE
Phage 1 (0)	-	-	-	-	-	-	-	-
Phage II (0)	-	-	-	-	-	-	-	-
Phage III (22)	0	59.1% (13)	77.3% (17)	86.4% (19)	100%	90.9% (20)	81.81% (18)	100%
Phage IV (2)	0	50% (1)	50% (1)	100%	100%	100%	100%	100%
Mixed I &III group (2)	0	50% (1)	50% (1)	100%	100%	100%	100%	100%
Mixed II & III Group (2)	0	50% (1)	50% (1)	100%	100%	100%	100%	100%
Non typable (64)	0	84.4% (54)	90.6% (58)	92.18% (59)	100%	90.63%(58)	81.25% (52)	100%

(P-Penicillin-Methicillin; CP-Ciprofloxacin; CfS- Cefaperazone- Sulbactum; V-Vancomycin; CD-Clindamycin; E-Erythromycin; TE- Teicoplanin.)

The present study showed similarity with Violet Kareiviene (2006) of phage III type predominance in MRSA but MSSA showed phage type II predominance. Phage group I & II showed total resistance to Penicillin in accordance with Samant and Pai (2012). Both typable and non typable strains showed total resistance to Penicillin and exhibited total sensitivity to Teicoplanin and Vancomycin in accordance with study of Suguneswari Giddi *et al.*, 2016.

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