A REPORT ON THE PROKARYOTIC BIO-LOAD SCALE AND OCCURRENCE OF *meca*$^{+}$ MRSA ON FOMITES FROM RESTAURANTS

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ABSTRACT

This work was carried with the objectives to calculate the bacterial count on the fomites of restaurants and to screen the fomite surfaces for the presence of mecA positive Methicillin Resistant Staphylococcus aureus (MRSA) strains. Two fomite surfaces that include eating table surfaces and hand washbasin surfaces of eight restaurants from educational institutions and commercial restaurants were screened to scale the bacterial counts. Colony counts were done using the dip slide technique. The results revealed that the tested eateries at the educational institutions harboured more bacterial colonies on the eating table surfaces than the hand washbasin surfaces. The reverse results of more bacterial colonies in the hand washbasin and less bacterial count on the eating table surface were observed in case of the fomites from commercial restaurants. Screening for the presence of Methicillin Resistant Staphylococcus aureus (MRSA) revealed that all the test fomite surfaces harboured MRSA. Multiplex PCR study showed that 37.5% of the isolated MRSA strains harboured mecA gene. Double disc diffusion test revealed that 25% of the MRSA isolates showed inducible Clindamycin resistance. The results highlight the existence of clinically significant pathogenic Staphylococcus aureus strains in the frequently accessible fomite surfaces of restaurants which could be a reservoir and portal for the establishment and dissemination of Community Acquired Methicillin Resistant Staphylococcus aureus infections.

Key Words: Fomites, mecA, MRSA, Inducible Clindamycin Resistance, PCR.

I. INTRODUCTION

Fomites – defined as inanimate objects that act as carriers of pathogenic microbes play a vital role in the establishment and dissemination of community acquired infections. Mostly fomite surfaces are colonized by secondary pathogens that are commensals on the skin of frequenting persons. Amongst the vast array of such pathogens, *Staphylococcus aureus* – a Gram Positive skin commensal and an opportunistic pathogen is a clinically significant organism. Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961 there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (Methicillin-Resistant *S. aureus*, MRSA). Since then MRSA isolates were isolated in many countries. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community. The methicillin resistance gene (*mecA*) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species (4). *mecA* is carried on a mobile genetic element, the Staphylococcal cassette

chromosome *mec* (*SCCmec*) [1]. Methicillin Resistant *Staphylococcus aureus* is an established nosocomial pathogen worldwide but more recently has emerged as a highly virulent organism in the community. Community Acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) infection is defined as illness compatible with Staphylococcal disease in a patient residing in the community and isolation of the organism from clinically relevant site [2]. CA-MRSA strains are transmissible from fomites to skin with transmissibility for many weeks after contamination [3].

In contrast to health care–associated MRSA (HA-MRSA), CA-MRSA is often susceptible to trimethoprim-sulfamethoxazole, clindamycin, doxycycline or minocycline, and Fluoroquinolones, although susceptibility to these agents may vary by geographic area. Given that the majority of reported CA-MRSA infections are skin and soft-tissue infections, Clindamycin represents an attractive option for several reasons. First, clindamycin comes in both intravenous and oral formulations (with 90% oral bioavailability). Second, the drug distributes well into skin and skin structures, and unlike ß -lactams, it is not impeded by a high bacterial burden at the infection site. Clindamycin is also less costly than some of the newer agents that might be considered for these infections. Finally, clindamycin may be able to inhibit production of certain toxins and virulence factors in Staphylococci. One of the major concerns with regard to the use of Clindamycin for CA-MRSA infection is the possible presence of inducible resistance to Clindamycin. The Erythromycin-Clindamycin D-zone test or the Double Disc diffusion test can separate strains that have the genetic potential to become resistant to Clindamycin during therapy from strains that are fully susceptible to Clindamycin [4].

II. MATERIALS AND METHODS

2.1 Calculation of Bacterial Bio-Load On Restaurant Fomite Surfaces

A total of eight restaurants that included 4 canteens from 2 educational institutions and 4 commercial restaurants in Madurai- Tamil Nadu were selected for the study. The fomite surfaces used in the study were the eating table surface and the hand washbasin sinks of each restaurants. Sampling of the surfaces were done using Hidip slides (Himedia). The two sides of the dipslide were pressed against the test surfaces for 5seconds according to the manufacturer's instructions. Results after incubation were read using the manual's reference chart [5].

2.2 Isolation and Identification of MRSA from the Fomite Surfaces

Detection of *mecA* gene appears to most accurately detect methicillin resistance in *Staphylococcus aureus*. It is known that many strains of methicillin resistant *Staphylococcus aureus* also demonstrate expression of Oxacillin resistance. As a result laboratory methods have been developed to enhance the expression of resistance in Staphylococci, including the supplementation of media with Nacl. The use of Oxacillin containing 6µgms of oxacillin per ml of the medium, as recommended by NCCLS, has been very useful for identifying Methicillin Resistant *Staphylococcus aureus* [6].

Sterile swabs in test tubes containing Nutrient broth (HiMedia) with 6µgms/ml Oxacillin and 4% Nacl were used to swab the fomite surfaces. The swabs were then incubated in the tubes for 48hrs. After the period of incubation, an aliquot of 100µls were quadrant streak inoculated in Mannitol Salt Agar plates (HiMedia). The plates were incubated at 37◦c for 24hrs. The golden yellow colonies were Gram stained. The isolates were then identified by tube coagulase test.Coagulase test was done by adding 0.2ml of broth culture to 0.5ml of plasma in a tube. It was mixed gently and incubated for 24hrs [7]. The isolates were subcultured in nutrient agar slants as MRSA isolates 1-16.

2.3 Multiplex PCR for *mecA* Gene Detection

All the isolated MRSA strains were subject to *mecA* gene detection by multiplex PCR study. The procedure was based on a modification by Unal *et al.*, (1992) [8] and this was used as the Gold Standard for all isolates. The oligonucleotides used were *mecA* F primer 1282 (5'- AAAATCGATGGTAAAGGTTGGC-3') AND *mecA* R primer 1793 (5'- AGTTCTGCAGTACCGGATTTGC – 3'), which gives a PCR product of 533bp [9].

2.4 Detection of Inducible Clindamycin Resistance

All the MRSA isolates were screened for the expression of inducible Clindamycin resistance (D Test) by placing a Clindamycin (2µgms) and Erythromycin (5µgms) disc adjacently with the disc centres 5-10mm apart on sterile Muller-Hinton Agar (HiMedia) plates swabbed with the test MRSA isolates [10].

III DISCUSSION

Analysis of the bacterial bioload scale on the test fomite surfaces revealed that the eating table surfaces of restaurants from educational institutions had a mean bacterial count of 10^5CFUs/cm^2, whereas the hand washbasin sinks had a mean bacterial load of 10^2CFUs/cm^2. Screening of the same fomite surfaces from commercial restaurants showed that the eating table surfaces had a mean bacterial load of 10^2CFUs/cm^2, while the hand wash basin sinks were colonized by a mean 10^5CFUs/cm^2. (Table-I)

An interesting feature in the study is that the eating table surface from educational institutions harboured comparatively higher bacterial counts than the washbasin surface. But the reverse result of lesser count on the eating table surface than the washbasin sink were observed in case of commercial restaurants.

Screening of the fomite surfaces for the presence of MRSA revealed that all the screened fomite surfaces harboured Methicillin Resistant *Staphylococcus aureus*. This finding highlights the definitive existence of a very clinically significant nosocomial pathogen in varying degrees of concentration at the most frequently contactable surfaces at the eateries (Table-II).

Molecular analysis of the isolated MRSA strains for the presence of *mecA* gene showed that an average of 37.5% of the isolates were only *mecA*$^+$. This could had been possible either by the fact that the rest of the isolates would have had exhibited an alternative protocol of methicillin resistance or were mecA$^-$ due to mutated alterations in the gene [11].

Analysis of the MRSA isolates by D test confirmed that 25% of the isolates had inducible Clindamycin resistance (Table-II). Interestingly, all the *mecA* positive MRSA isolates and isolates that exhibited positive D test were from the fomites of commercial restaurants only and that no inducible Clindamycin resistant MRSA strains or *mecA*$^+$ strains were detectable on the fomites from educational institutions despite our low test samples. This could perhaps be attributed to the type of frequenters to the restaurant and their personal hygiene.

IV. CONCLUSION

The study confirms the existence of varying degrees of microbial load and the presence of Methicillin Resistant *Staphylococcus aureus* on the fomite surfaces of eateries. Presence of such a clinically potential pathogen confirms that commercial fomites serve as reservoirs for various pathogenic bacteria that are potent to initiate community acquired infections.

Table I: Bioload Count of the Test Fomite Surfaces

Restaurant	Fomite Location	CFUs/cm^2
College-I Canteen-I	Eating Table Surface	10^6
	Wash Basin Sink	10^2
College-I Canteen-II	Eating Table Surface	10^5
	Wash Basin Sink	10^3
College-II Canteen-I	Eating Table Surface	10^5
	Wash Basin Sink	10^3
College-II Canteen-II	Eating Table Surface	10^5
	Wash Basin Sink	10^2
Commercial Restaurant-I	Eating Table Surface	10^3
	Wash Basin Sink	10^6
Commercial Restaurant-II	Eating Table Surface	10^3
	Wash Basin Sink	10^6
Commercial Restaurant-III	Eating Table Surface	10^3
	Wash Basin Sink	10^5
Commercial Restaurant-IV	Eating Table Surface	10^2
	Wash Basin Sink	10^4

Table II: Source, Biochemical, Molecular and Clinical Characteristics of The Isolated MRSA Strains

Restaurant	Fomite	MRSA Isolate	Coagulase	*mecA*	D Test
College-I Canteen-I	Eating Table Surface	1	+	-	-
	Wash Basin Sink	2	+	-	-
College-I Canteen-II	Eating Table Surface	3	+	-	-
	Wash Basin Sink	4	+	-	-
College-II Canteen-I	Eating Table Surface	5	+	-	-
	Wash Basin Sink	6	+	-	-
College-II Canteen-II	Eating Table Surface	7	+	-	-
	Wash Basin Sink	8	+	-	-
Commercial Restaurant-I	Eating Table Surface	9	+	+	-
	Wash Basin Sink	10	+	+	+
Commercial Restaurant-II	Eating Table Surface	11	+	-	-
	Wash Basin Sink	12	+	+	+
	Eating Table Surface	13	+	-	-

Commercial Restaurant-III	Wash Basin Sink	14	+	+	+
Commercial Restaurant-IV	Eating Table Surface	15	+	+	-
	Wash Basin Sink	16	+	+	+

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