Original Article

Preoperative screening for mucocutaneous bacterial colonization with special reference Methicillin Resistant Staphylococcus aureus

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Preoperative infection control policy depending on local prevalence of bacterial colonization in order to reduce postoperative surgical site infection is a vital concern of any health care setting. The aims of the present study were to determine the local prevalence of mucocutaneous colonization of Methicillin resistant Staphylococcus aureus (MRSA) and other bacteria in patients presenting for emergency and elective surgery, to identify patient groups mostly colonized and the most frequent colonizing bacteria, to determine the sensitivity of MRSA screening of nose, throat, axilla, groin and perineum individually and to compare the individual site sensitivities with the sensitivity of multiple site screening. Cross-sectional study in a tertiary public teaching hospital. Active surveillance of 400 patients was done preoperatively to detect mucocutaneous bacterial colonization from five sites namely nose, throat, groin, axilla and perineum. Bacterial colonization was detected in the nose, throat, axilla, groin and perineum in 53.5%, 50%, 86%, 76.3% and 76.8% of preoperative patients respectively. The most frequent bacteria isolated from nose, throat, axilla and perineum was Methicillin Sensitive Staphylococcus aureus (MSSA). In the groin, MRSA was most frequent. The prevalence of MRSA in the study population was 45.25%. Preoperative bacterial colonization was most prevalent in the age group between 21 and 40 years. Females were more colonized than males. Multiple site screening increased the sensitivity for MRSA detection when compared to single site screening. MRSA colonization was mostly found in the groins of preoperative patients. Culturing multiple sites was superior to culturing any single site for detection of MRSA colonizers.

[*J Indian Med Assoc* 2018; **116:** 7-12]

Key words **: Preoperative, mucocutaneous, bacterial, colonization, MRSA.**

Postoperative surgical site infections (SSIs) are a major factor in the morbidity and mortality in patients undergoing elective or emergency surgery. Staphylococcus aureus is the commonest cause of SSIs, of which the methicillin resistant strain is most threatening[1]. Preoperative colonization of the skin is an important cause of SSIs. Methicillin Resistant Staphylococcus aureus (MRSA) colonized patients are at a higher risk of infection than non-MRSA-colonized patients[2]. Several virulence factors increase both the preponderance and the severity of MRSA related SSI.

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- **Preoperative infection control is the key to reduce postoperative surgical site infection.**
- **Preoperative bacterial colonization is most prevalent between 21 and 40 years.**
- **Females are more commonly colonized than males.**
- **Groin is most frequently colonized with MRSA.**
- **Multiple sites culturing is superior to detect MRSA colonizers.**

Previously, MRSA primarily affected hospitalized or chronically ill patients with implanted devices or catheters or patients living in long-term care facilities such as nursing homes. However, more recently MRSA has been found in patients in the community, who do not share the same associated risk factors as patients in hospital[3].

There has been a tremendous increase in community-acquired MRSA (CA-MRSA) a subpopulation of MRSA with unique antibiotic resistance properties, virulence characteristics and pathogenic capability. As a cause of SSI, Methicillin resistant Staphylococcus aureus (MRSA) has been associated with higher rates of patient morbidity and

mortality than the methicillin-sensitive strain. This morbidity and mortality increases healthcare costs.

In order to reduce the incidence of MRSA infection, universal patient screening has been advocated by many studies. Several studies have evaluated the efficacy of screening patients for MRSA colonization via traditional culture-based techniques and rapid detection via polymerase chain reaction (PCR)[4].

The sensitivity and specificity of PCR is 100% and 98%, compared with sensitivity and specificity of 90% and 100% for culture. Real-time PCR yields results within 24 hours, whereas standard cultures may take up to three to four days[5]. However, the main disadvantage of PCR is the cost, which make it limited importance in developing country.

The standard regime for screening by cultures is a 5 site sampling method including nose, throat, axilla, groin and perineum. But some studies have shown that culturing fewer sites may be equally effective while reducing costs, time to obtain results and laboratory workload in busy, often resource limited, hospitals[6].

Although there are Indian studies on ICU colonization, there are very few data on preoperative elective or emergency surgery patients. The first step to understand the magnitude of the problem of preoperative colonization and to decide further studies to evaluate the efficacy of any decolonization programme, if implemented, is to obtain local epidemiological data on MRSA (or any other bacteria) colonization[7].

Therefore, the objective of this study was to get local data about prevalence of MRSA (and other organism ,if any) mucocutaneous colonization, to identify patient groups who are mostly colonized, to identify the most frequent colonizing bacteria in those colonized patients, to determine the sensitivity of MRSA screening of nose, throat, axilla, groin and perineum individually in patients presenting for emergency and elective surgery and to compare it with the sensitivity of multiple site screening.

Material and Methods

This cross-sectional study was conducted in the department of Anaesthesiology and department of Microbiology of our tertiary teaching hospital over a 12 months' period (October 2014 to September 2015) on preoperative patients selected for elective or emergency surgery.

Recent studies indicate that MRSA is endemic in our country, with an isolation rate of nearly 27%, 49% and 47% amongst clinical isolates of Staphylococcus aureus from out-patients, ward-in-patients and intensive care unit (ICU) patients respectively[8].

Estimation of the prevalence of bacterial colonization was taken as the primary research question for the purpose of sample size calculation. It was estimated that 380 samples would be needed to be analyzed in order to estimate the prevalence at any one site with a 5% margin of error and 95% level of confidence. Keeping 5% allowance for non-analyzable or lost samples, the sampling target was fixed at 400 samples per site.

All preoperative patients selected for surgery who themselves had consented for screening were included in the study. Incompetent patients whose guardians or next of kin gave written informed consent were also included. Patients or relatives not consenting for screening were excluded.

After obtaining written informed consent from the patient, guardian or next of kin, swabs were taken from five different sites, namely, anterior nares, throat, axilla, groin and perineum. The swabs were immediately submitted to the department of Microbiology.

For patients presenting for elective surgery, swabs were taken during the pre-operative assessment in the Pre-Anesthetic Checkup (PAC) outpatient clinic. For patients undergoing emergency surgery, the samples were collected just before surgery.

In the Microbiology department, the samples were inoculated on Blood Agar, Mac- Conkey Agar, Nutrient Agar and/or Chocolate Agar and incubated at 37°C for 18-24hours. Inoculation on Mannitol Salt Agar (MSA) was done for isolation of "might be pathogenic" bacteria e.g. staphylococcus, acinetobacter etc. Organism identification was subsequently done by Gram stain, colony morphology, motility and relevant biochemical tests. Antibiotic sensitivity tests were done as per Central Laboratory Standards Institute (CLSI) guidelines[9].

Colonies suggestive of Staphylococcus aureus were further identified by Gram stain, catalase, and slide and tube coagulase. The isolates were confirmed as MRSA by disc diffusion test using 30 microgram cefoxitin disc on Mueller Hinton agar, as per Clinical Laboratory Standards Institute CLSI recommendations[9].

Statistical analysis: The collected data were entered in Microsoft excel worksheet (Microsoft, Redwoods, WA, USA) and checked for accuracy. Quantitative data were expressed in percentages.

Results

More than half of preoperative patients going for elective or emergency surgery were found to have bacterial colonization in one or more of the screening sites. The axilla (344/400, 86% of patients) was the most frequently colonized site, followed by perineum (307/400, 76.8 %) and groin (305/400, 76.3%). Bacterial colonization was lesser in the nose (214/400, 53.5%) and throat (200/400, 50%). (Table 1). The nose was the most frequent site found to have no bacterial growth (200/400, 50%).

Methicillin sensitive Staphylococcus aureus (MSSA)

Table 1 — *Frequency of bacterial growth in each sampling site*

	Nose	Throat	Axilla	Groin	Perineum
Growth present	214 (53.5 %)	200 (50.0 %)	344 (86.0 %)	305 (76.3 %)	307 (76.8 %)
No growth	186 (46.5 %)	200 (50.0 %)	56 (14.0 %)	95 (23.8 %)	93 (23.3 %)
Total	400 (100 %)	400 (100 %)	400 (100 %)	400 (100 %)	400 (100 %)

was the most frequently isolated colonizing bacteria in all the screening sites except the groin, where MRSA was mostly isolated. Axilla, the most common site of bacterial colonization, had the highest percentage of MSSA colonization (35.8%) amongst all microorganisms. MRSA, the most ominous threat, was found in 19.8% isolates grown from groin (Table 2).

Table 2 — *Most frequent bacteria isolated from each sampling site*

Site	Most frequent bacteria isolated
Nose	MSSA (26.8%)
Throat	MSSA (14.2%)
Axilla	MSSA (35.8%)
Groin	MRSA (19.8%)
Perineum	MSSA (23.8%)

MSSA: Methicillin sensitive staphylococcus aureus, MRSA: Methicillin resistant staphylococcus aureus

Besides the most frequent colonizers viz MSSA and MRSA, other bacteria isolated from the various sites included Gram negative bacteria e.g. Acinetobacter, E coli, Klebsiella, Proteus, Citrobacter and Pseudomonas too. The relative percentages of each colonizing bacteria in the five screening sites are tabulated in Table 3.

In the present study, multiple site screening for colonization of MRSA was performed on 400 patients, out of which 181 patients had carriage of MRSA at one or more site, giving an overall positivity of 45.25%. Sensitivity of MRSA detection from culture of a single anatomical site was found to be lower than that from simultaneous culture of multiple sites. Sensitivity of MRSA detection from only nose was 21.54%, only throat was 23.20%, only axilla was 41.43%, only groin was 43.63%, and only perineum was 25.96% (Table 4).

Table 4 — *Positivity rates of single sampling sites and their sensitivities for MRSA*

Single site	Number with positive results (n=181)	Percentage of probability of positivity/Sensitivity
Nose	39	21.54
Throat	42	23.20
Axilla	75	41.43
Groin	79	43.64
Perineum	47	25.96

The sensitivities of MRSA detection were found to be higher when two anatomical sites were combined and further higher when three anatomical sites (throat, axilla and groin) were combined (81.76%) (Table 5).

Table 5 — *Positivity rates of combinations of sampling sites and their sensitivities for MRSA*

Combination of sites	Number with positive results (n=181)	Percentage of probability of positivity/ Sensitivity
Nose+Throat	72	39.77
Nose+Axilla	97	53.59
Nose+Groin	106	58.56
Nose+Perineum	79	43.64
Throat+Axilla	103	56.90
Throat+Groin	115	63.53
Throat+Perineum	82	45.30
Throat+Axilla+Groin	148	81.76

In the present study, preoperative patients between 21 and 40 years were found to be mostly colonized by bacteria. Females were more colonized than males.

Discussion

The issues regarding colonization versus infection of hosts by microorganisms are often poorly understood. MRSA, which is a resistant strain of Staphylococcus aureus, can reside on human skin for long periods or even permanently. Colonization indicates the presence of an organism without symptoms of illness. It can occur in the nares, trachea, skin folds, rectum, or in an open wound such as decubitus ulcer and is applicable for any microorganism. On the contrary, infection is defined as tissue invasion by a microorganism with subsequent clinical symptoms. Regarding carriage, all MRSA carriers are not the same and carriage may be transient, intermittent, or persistent for months to years[10,11,12]. Persistent carriers are more heavily colonized (frequently at multiple sites), are more likely to transmit the strain to others, and are more prone to become infected than transient carriers[12]. About 60% of the general population are intermittently colonized and about 20% are persistently colonized in the anterior nares with methicillin sensitive or resistant strains of Staphylococcus aureus[11]. MRSA usually prefers to harbor in the warmest, dampest and darkest areas of the body e.g. the nose, throat

Table 3 — *Type of bacteria isolated from each sampling site*

	Nose	Throat	Axilla	Groin	Perineum
MSSA	107 (26.8 %)	57 (14.2 %)	143 (35.8 %)	78 (19.5 %)	95 (23.8 %)
MRSA	39 (9.8 %)	42 (10.5 %)	75 (18.8 %)	79 (19.8 %)	47 (11.8 %)
MSCONS	22 (5.5 %)	16 (4.0 %)	29 (7.2 %)	41 (10.3 %)	25 (6.3 %)
MRCONS	32 (8.0 %)	9 (2.3 %)	50 (12.5 %)	43 (10.8 %)	37 (9.3 %)
Acinetobacter	5 (1.3 %)	15 (3.8 %)	5 (1.3 %)	4 (1.0 %)	8 (2.0 %)
E.coli	3 (0.8 %)	8 (2.0 %)	17 (4.3 %)	24 (6.0 %)	57 (14.2 %)
Klebsiella	6 (1.5 %)	52 (13.0 %)	25(6.3 %)	32 (8.0 %)	27 (6.8 %)
Pseudomonus	0 (0.0 %)	1 (0.3 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Citrobacter	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	4 (1.0 %)	0 (0.0 %)
Proteus	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	11 (2.8 %)
No Bacteria	186 (46.5 %)	200 (50.0 %)	56 (14.0 %)	95(23.8 %)	93 (23.3 %)
Total	400(100 %)	400(100.0 %)	400(100.0%)	400(100.0%)	400(100.0 %)

(MSSA: Methicillin sensitive staphylococcus aureus, MRSA: Methicillin resistant staphylococcus aureus, MSCONS : Methicillin sensitive coagulase negative staphylococcus, MRCONS: Methicillin resistant coagulase negative staphylococcus)

or armpits although they can reside virtually on any skin surface. It is widely accepted that the nose represents the primary reservoir of Staphylococcus colonization in humans. The throat has emerged as another important area while all other sites are considered secondary. However, nasal carriage has not been universally found among MRSA positive patients with implanted devices, and the rectum may be an important reservoir among those with community acquired MRSA[13,14]. Therefore, if the goal of an intervention is true eradication of MRSA carriage, then routine surveillance by culture or molecular methods should better involve more than one sites in order to detect all colonized patients. So the natural question arises regarding the selection of sites which need to be targeted for surveillance screening and eradication of MRSA colonization and if colonization is found, what would be the next step? Our study focuses in the perioperative setting and hence the first question has been addressed pertaining to preoperative patients selected for emergency or elective surgery in the preset report.

The main complication of MRSA colonization in the perioperative setting is surgical sites infection (SSI). Hence the need diagnosis and if necessary, eradication of significant colonization in preoperative patients is important. Some groups have advocated "search and destroy" policies which recommend routine screening for MRSA to identify, isolate, and treat carriers, with the ultimate goal of eradicating the pathogen from health care facilities[15]. But if this policy is implemented, natural questions arise regarding its efficacy, cost effectiveness and applicability in the preoperative patient group including the appropriate decolonization protocol in this group of patients.

Once Staphylococcus aureus colonization has been established, preoperative patients are commonly treated with intranasal mupirocin ointment twice a day for 5 days prior to surgery[16,17] often supplemented with daily topical chlorhexidine washes for 5 days prior to surgery using a washcloth or a scrub. The use of rifampin ensures excellent penetration into secretions and tissues. Another add on strategy is to use preoperative antibiotics according to the susceptibility report of colonization testing on the day of surgery (like cefazolin alone for MSSA colonized & vancomycin alone for MRSA colonized). However, development of drug resistance and the loss of valuable therapeutic agents for subsequent treatment of infection is a big issue for the wide scale use of systemic antibiotics. These decolonization issues for any microorganism can only be properly if we can first diagnose colonization in the most comprehensible manner, for which, surveillance site selection is the primary job. Our study addresses this particular issue of site selection.

Infections at or near surgical incisions within 30 days of an operative procedure contribute to surgical morbidity and mortality. The prevention of surgical site infections encompass meticulous operative technique, timely administration of appropriate preoperative antibiotics and a variety of preventive measures aimed at neutralizing the threat of bacterial, viral, and fungal contamination posed by operative staff, the operating room environment, and the patient's endogenous skin flora.

Postsurgical infection leads to increased length of postoperative hospital stay, drastically escalated expense, higher rates of hospital readmission, and jeopardized health outcomes. Surgical site infections, accounting for 15% of all nosocomial infections and among surgical patients, represent the most common nosocomial infections[18].

Surgical site infections raise costs due to prolonged hospitalization, additional diagnostic tests, therapeutic antibiotic treatment, and rarely additional surgery[19]. In the majority of SSI cases, the pathogen source is the native flora of the patient's skin, mucous membranes, or hollow viscera[20]. When skin is incised, underlying tissue is exposed to overlying endogenous flora[21]. Most typically, aerobic gram positive cocci such as staphylococcus serve as the contaminant, with resistant pathogens like MRSA representing an increasing proportion of such infections in recent years[22,23]. Entry into hollow viscera exposes surrounding tissue to gram-negative bacilli such as Escherichia coli, gram-positive organisms such as enterococcus, and, occasionally, anaerobes such as Bacteroides fragilis too. Yeast species and viral pathogens also pose a risk[24]. Among the Gram positive pathogens, Staphylococcus aureus continues to cause skin and soft tissue infections (SSTI) in the community as well as invasive infections in the hospitalized patients. In a recent Europe-wide survey, the most common organisms in SSTIs were Staphylococcus aureus (71% cases) with 22.5 per cent being MRSA[25].

In our study, MSSA was the most frequent colonizing bacteria in nose (26.8% patients), throat (14.2%), axilla (35.8%) and perineum (23.8%), whereas in groin, the most frequent bacteria isolated was MRSA (19.8%). MSSA was found in groin of 19.5% patients. Our finding of Staphylococcus aureus as the most frequent colonizer was identical with that of Naik *et al*[26]. Our most frequent age group of patients colonized (21-40 years) was also similar to that in the referred study[26]. But females were more commonly found to be colonized in our study in contrary to the findings of Naik *et al*[26], where males are more frequent colonizers.

The prevalence of MRSA varies between regions and between hospitals in the same region. In a three year study by Ranjan KP *et al*[1], Staphylococcus aureus (34%) was the most common isolate from surgical site infections, of which 27.96% were found to be methicillin resistant and

the isolates were mostly demonstrated from patients between 21 and 40 years and males. Mehta *et al*[27] also reported 33% prevalence of MRSA in their study; Hussain *et al*[28] reported a 65% prevalence of MRSA. The prevalence of MRSA in a study from Chennai[29] was reported as 40-50 per cent. In a study from north India[30], the prevalence of MRSA was 46% .As per Indian Network for Surveillance of Antimicrobial Resistance (INSAR) Group, India, recent studies indicate that MRSA is endemic in our country, with an isolation rate of nearly 27%, 49% and 47% amongst clinical isolates of Staphylococcus aureus from outpatients, inpatients and ICU patients respectively[8]. Our study revealed a prevalence of MRSA was 45.25% and although, our data relate to a specific group of patients ie, preoperative patients selected for elective or emergency surgery, our results are in keeping with contemporary Indian data on MRSA.

L Senn *et al*[31] in 2012 observed that a significantly higher proportion of MRSA cases were detected with groin specimens. Similarly, in our study, the anatomical site which most often yielded positive result was groin swab (43.64%) followed by axilla swab (41.43%). Datta P *et al*[32] compared different anatomical sites, either alone or in combination, for screening MRSA carriage in the ICU on 400 adult patients in a multi-disciplinary tertiary care ICU. Their results revealed an overall MRSA carriage rate of 22.5%. Amongst the six sites tested for detection of MRSA carriage, sampling from throat alone exhibited a sensitivity of 84.4%, followed by that from nose (77.7%), groin (55.5%), perineum (40%), axilla (33.3%) and the site of catheterization (17%). Multiple sampling further improved the detection sensitivity, with a combination of the nose and throat swabs detecting the maximum MRSA carriers (95.5%). Likewise, combining throat and groin swabs or nasal and groin swabs also yielded higher detection sensitivity (93.3% and 91.1% respectively) compared with single-site sampling. Based on these data, they reported that throat was the most common site of MRSA colonization and nose or groin should be sampled simultaneously for superior detection sensitivity. Classically, anterior nares have been considered the major anatomical site for Staphylococcus aureus screening. Many newer studies indeed suggest that including swabs from colonization sites other than the anterior nares increases the MRSA screening sensitivity. In contrary to the above-mentioned study[32], our study on preoperative patients revealed that the sensitivity of MRSA detection from preoperative culture of single anatomical site was low. Sensitivity of MRSA detection from only nose was 21.54%, throat 23.20%, axilla 41.43%, groin 43.63% and perineum 25.96%. When two anatomical sites were combined in our study, the sensitivity of detection of colonization increased and when more than two sites were combined, the sensitivity increased significantly to more than 80%.

Our study has limitations. Being a single centre study on preoperative patients, the results cannot be extrapolated on all types of patients across all categories health facilities. Individual studies from different institutions may provide institution-specific colonization data, which may help in planning specific decolonization measures or planning antimicrobial protocols, if needed. Multicentre regional studies may also add information about colonization trends in a particular geographic or socioeconomic area. Our study has focused on the prevalence of MRSA colonization only and has neither dealt with the deleterious effects, if any, of such colonization, nor the efficacy of any decolonization measure. Further researches are needed to evaluate the effects of preoperative colonization on postoperative infective complications and also to study the efficacies of decolonization measures in surgical patients.

Conclusion

In conclusion, our study on preoperative elective or emergency surgical adult patients revealed that patients between 21 and 40 years were found to be most frequently colonized by bacteria and that females were more commonly colonized than males. On culturing multiple sites, it was found that selected that nose, throat, axilla and perineum were mostly colonized by MSSA whereas the groin was mostly colonized by MRSA. The overall prevalence of MRSA colonization among the preoperative patients was 45.25%. Besides MSSA and MRSA, other colonizing bacteria included Methicillin sensitive coagulase negative staphylococcus (MSCONS), Methicillin resistant coagulase negative staphylococcus (MRCONS), Acinetobacter, Escherichia coli, Klebsiella, Proteus, Citrobacter and Pseudomonas. The study also showed that culturing any single anatomical site was insufficient for efficient detection of MRSA carriers. On the contrary, the sensitivity for MRSA detection increased when multiple anatomical sites were screened.

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