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Screening of Methicillin Resistant *Staphylococcus aureus* Isolates by Different Advanced Techniques in Hospital Settings.

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ABSTRACT

Methicillin- Resistant *Staphylococcus aureus* (MRSA) isolates are a serious public health concern and its ever-increasing rate is exerting pressure on the healthcare system. At present, more than 20 % of clinical *S. aureus* isolates in tertiary care hospitals are methicillin resistant. Asymptomatic colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) has been described as a risk factor for subsequent MRSA infection, hence nasal screening study was carried out to determine clinical effectiveness of polymerase chain reaction as compared to chromogenic agar and conventional microbiological culture. Double swabs were used for nasal sampling, the first swab was used to run the GeneXpert MRSA assay and the second one was inoculated on to CHROM agar, blood agar and chocolate agar for MRSA isolation. Identification and antimicrobial susceptibility testing were performed. Nasal specimens (2347 numbers) were screened using Xpert MRSA. Among the 2347 nasal swabs screened, 265 swabs (11.29 %) proved to be positive for MRSA amplifications. Among them, 1928 (82 %) were negative while 154 (6.56 %) specimens were unresolved for the presence or absence of MRSA. Out of 154 unresolved specimens, only 3 (1.9 %) of them showed positive in CHROM agar and conventional culture methods while the remaining possessed heavy mixed growth and Pseudomonas colonization. The results of GeneXpert MRSA was found to be similar in efficiency to conventional culture methods, but the advantage of the technique is that it consumes lesser time for each sample so that more number of samples could be analyzed in a short time. The GeneXpert System has reduced the TAT of MRSA screening from 2-3 days down to 75 min and has eliminated the need of presumptive isolation of patients. This particular study has helped to reduce nursing labor, improved bed management and has reduced isolation costs in the ICU.

Keywords: *Staphylococcus aureus,* resistance, antibiotics, microbial culture

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the most common bacterial pathogen isolated in cases of severe infection in both hospital and outpatient medical care. Since its first description in the early 1960s, Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of health care acquired infection [1]. Twenty years later, a mobile genetic element (Staphylococcal Cassette Chromosome mec (SCC mec) which was integrated into the *S. aureus* chromosome, was identified. It is a specific genetic mechanism of methicillin resistance. Here, the mecA gene, encoding a specific methicillin-resistant trans peptidase (penicillin-binding protein 2a) confers resistance to all Beta-lactam antibiotics. Thus the inhibition of native pBp has been overcome by these antibiotics [2]. This has been the reference standard for the detection of MRSA. Eleven different types of SCC mec elements have been defined for MRSA (1 to 11), [3]; IWG-SCC., 2009). Early identification of patients with MRSA nasal carriage can be part of an effective infection prevention program, [4]. According to the findings of the hospital surveillance program of nosocomial infections, 18 % of the 60,000 hospital infections that occur each year in intensive care are due to *Staphylococcus aureus* [5]. Of these, 20-25 % of the clinical isolates are methicillin-resistant. This is alarming because methicillin resistance in *S. aureus* not only means limited effectiveness of antibiotic treatment, but also leads to prolonged hospital stay and higher morbidity and mortality rates, [6]. Large outbreaks of MRSA in other hospitals, and among otherwise healthy individuals in the community [7] raise the concern that this organism is spreading outside its traditional role as a health care –related pathogen, hence early detection method is the need of the hospital. The use of surveillance cultures greatly improves the detection of MRSA colonization compared to clinical cultures alone [8].

Screening of cultures for MRSA on-admission is one of the mainstays for the successful 'search and destroy' infection control policy in our hospital. Despite the fact that culture-based MRSA screening swabs have proven to be cheap, sensitive and practicable, the delay between sample acquisition and reporting of results remains a significant drawback. Reliable identification and testing results are usually available only 48–96 h after sample collection, and during this time MRSA cross-transmission could occur if patients are not placed under contact precautions ('precautionary isolation') [9, 10]. As these measures may be unnecessary or, if not applied, unidentified MRSA-positive individuals may remain a hidden reservoir for cross-transmission, the need for speedier methods to detect MRSA is widely acknowledged. In hospitals, transmission occurs from a colonized or an infected individual to others, mainly via the hands of transiently-colonized healthcare workers [11]. MRSA has been associated with many infection sites, including bones and joints, lungs, and the urinary tract. Bacteremia is common, possibly leading to endocarditis and osteomyelitis [12].

Methicillin resistant *Staphylococcus aureus* (MRSA) are often resistant to multiple classes of antibiotics. It has also been reported that the speed with which MRSA carriage is detected has an important role to play, as it is a key component of any effective strategy to prevent the pathogen from spreading. Since MRSA culturing involves a 2–3 day delay before the final results are available, rapid detection techniques (commonly referred to as 'MRSA rapid tests') using PCR methods and, most recently, rapid culturing methods have been developed. The Xpert MRSA assay (Cepheid, Sunnyvale, CA), which runs exclusively on the GeneXpert system [13] is a sample –in /answer –out tests. The implementation of rapid tests MRSA carriers has reduced the timings from 72 h to 75 min [14, 15]. However, PCR-based methods require concomitant cultures to recover organisms for epidemiological typing or further susceptibility testing. Clinical evaluation data have shown that MRSA can thus be detected with very high sensitivity and specificity. However it is sometimes impaired due to false-positive PCR signals occurring in mixed flora specimens. In order to rule out any false-positive PCR results, a culture screen was carried out simultaneously.

While both molecular and culture techniques have primarily focused on the detection of MRSA, recent data also suggest in certain populations that both MRSA and methicillin -sensitive S*taphylococcus aureus* (MSSA) should be part of the screening [16].

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MATERIALS AND METHODS

Patient Population

Nasal swab (n=2347) which were received in the clinical bacteriology laboratory from inpatient of 650 beds tertiary care hospital with 68 ICU beds, from January 2013 to December 2014 were included in the study. Nasal samples were obtained for culture at admission and during hospitalization. Patients were excluded from the study if they had received treatment with intranasal mupirocin in the previous 14 days or treatment with oral antimicrobials for the purpose of eradicating MRSA colonization within the past 14 days had contraindication to nasal sampling.

Specimen collection, Transport and Storage

All specimens were collected by nursing personnel from the tertiary care hospital. Specimens were transported to our laboratory by a pneumatic tube transport system and performed immediately.

Culture media

CHROM agar plates for MRSA were obtained from Medisinal, (manufactured in association with Becton Dickinson), and for the standard culture Blood agar, Chocolate agar and Macconkey agar were used. Chromogenic media were stored in the dark prior to inoculation and during incubation for this study. Quality control testing was performed on each chromogenic medium and conventional culture medium daily using a standardized inoculum of *S. aureus* ATCC 25923.

Study design

Work flow:

- A total of 2347 nasal swabs were tested in this study. One nasal swab from the double swab collection and transport system was used to run in the GeneXpert MRSA assay and the second one is inoculated on to CHROM agar, blood agar and chocolate agar for the isolation of MRSA, then they were compare. Identification of presumptive colonies was confirmed by DNAase test and MRSA was confirmed by the cefoxitin disk test (Figure 4).
- *Inoculation and incubation:* Swab was first rotated then plated onto CHROM agar, BA, and CA in a randomized order. Plates were streaked for isolation, and all plated media were incubated at 37° C for 24 h in a dark incubator, with examinations at 24 and 48 h (Figure 1).
- (*iii)Xpert MRSA* - The newly developed assay is called 'Xpert MRSA' and is performed on a closed, self-contained, fully integrated and automated platform (Gene Xpert DX instrument; Genzyme Virotech), which represents a paradigm shift in the automation of molecular analysis, producing accurate molecular results on demand. Xpert MRSA Assay is a qualitative in vitro diagnostic test designed for direct detection of MRSA nasal colonization.

Detection of MRSA

By conventional method

All *S. aureus* isolates were confirmed by using the Staphaurex latex agglutination test, DNAase and antimicrobial susceptibility testing. Antimicrobial Susceptibility testing was done, by using automated microbiology systems (VITEK 2) and manually by applying oxacillin E test strip and cefoxitin disk in Mueller Hinton agar (Figure 3 and 4). Plates were incubated at 37 °C for 18 to 24 h and examined for evidence of growth. The most accurate phenotypic test for the presence of the *mecA* gene in *S. aureus* is the cefoxitin disk diffusion test. Prepared a lawn culture of the test isolate on Mueller Hinton agar +2 % sodium chloride. Applied cefoxitin disk (30 µg) and

incubated for 24 h at 37º C. According to the Clinical and Laboratory Standards Institute (CLSI), a zone of growth inhibition around the cefoxitin disk of ≥ 22 mm rules out MRSA; a zone size < 22 mm indicates that the *mecA* gene is present and the isolate should be reported as MRSA (Wayne, 2012). Cefoxitin is used because it is a more potent inducer of *mecA* expression than other agents such as oxacillin and the test results are relatively easy to interpret.

Chromogenic culture interpretation:

Rapid culture makes use of chromogenic agar, which contains media substrates that change color in the presence of *S. aureus*; selectivity for MRSA is achieved by incorporation of antibiotics into the agar. Use of such agar grows pink colored colonies (Mauve colonies characteristic for MRSA), *i.e.*, identification of MRSA from primary isolation plates within 24 to 48 h, obviating the need for additional subcultures or biochemical tests [4].

By PCR Method

The polymerase chain reaction (PCR) is a molecular technique in which enzymatic replication is used to amplify a short sequence of DNA. It is used to reproduce selected sections of DNA and the presence of MRSA is more rapidly and easily detected compared with culture-based methods, which can take one to two days [17]. PCR is used to identify the SCC mec cassette that contains the mecA gene and orfX, an opening frame distinctive to *Staphylococcous aureus* [18, 19]. To further reduce the time from screening to notification of test results, the POCT (point-of-care testing) concept has very recently been applied to MRSA testing.

RESULTS

Nasal swab specimens were obtained from 2347 patients 265 (11 %) were positive by MRSA by Xpert MRSA assay while 1,928 (82 %) samples were MRSA negative. 154 (6.56 %) specimens were reported as error by PCR. Only 3 of 154 (1.9 %) error swab showed positive in CHROM agar and conventional culture methods, remaining error swab shows heavy mixed growth and some shows pseudomonas colonization. In PCR reactions, 265 (11%) out of 2347 were positive, but with culture methods 268 (11.4%) were positive. In both PCR and culture 1928 (82%) were negative. Subsequently, in PCR 154 OUT OF 2347 were unresolved. The unresolved PCR result repeated in culture showed that 151 out of 154 had heavy mixed growth. The overall sensitivity was 98.8% and specificity was 100%. The TAT by Xpert MRSA is 75 minutes which is substantially lower than conventional culture which has a TAT of 2-3 days. Our study reported that the turnaround time for test results and number of isolation days was lower for PCR versus chromogenic agar and conventional methods for MRSA screening. According to previous reports, from the year 2006, MRSA results in non-ICU wards were getting declined after the introduction of molecular methods. Unfortunately, in the year 2012, PCR methods were stopped due to administrative reasons. Again in the year of 2013 and 2014 MRSA results in all wards including ICU were reduced after the introduction of GeneXpert (Figure 5).

Samples processed by the CHROM agar method were 1.49 times more likely to acquire MRSA compared to those in the PCR method. The study also found that the MRSA incidence rate to be lower for patients who were screened using PCR compared to patients who were screened using chromogenic agar.

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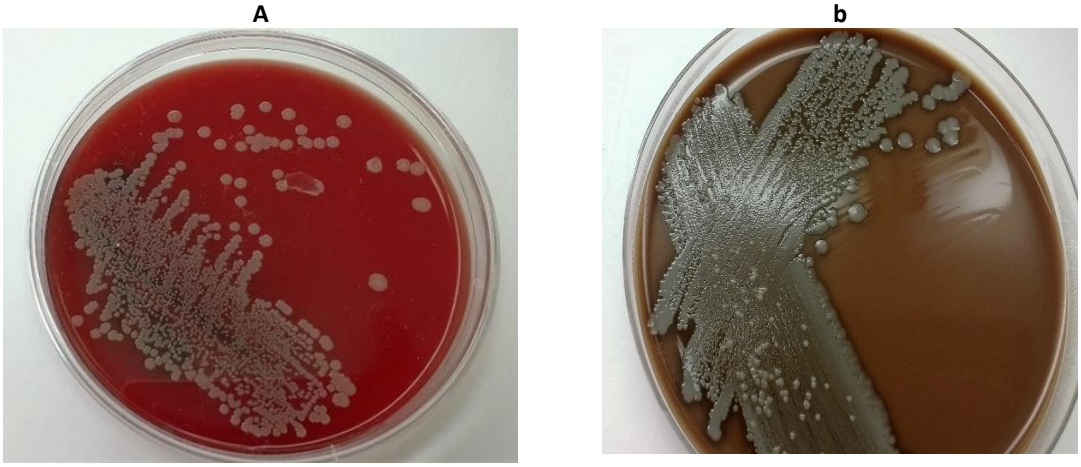

Figure 1. Conventional culture method. Colonies of MRSA in (a) Blood agar and (b) Chocolate agar.

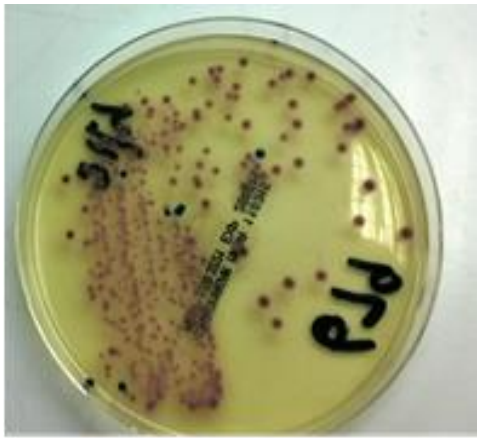

Figure 2: MRSA in chromogenic media

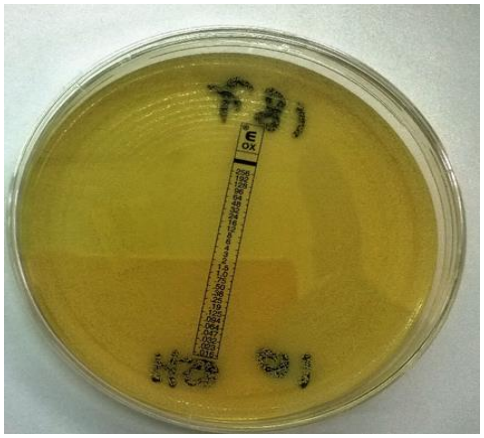

Figure 3: Oxacillin E test strip resistant

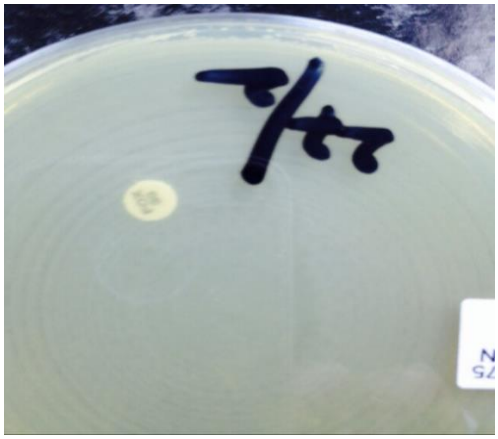

Figure 4: Cefoxitin disc diffusion test to detect MRSA

Figure 5: GENEXPERT-PCR

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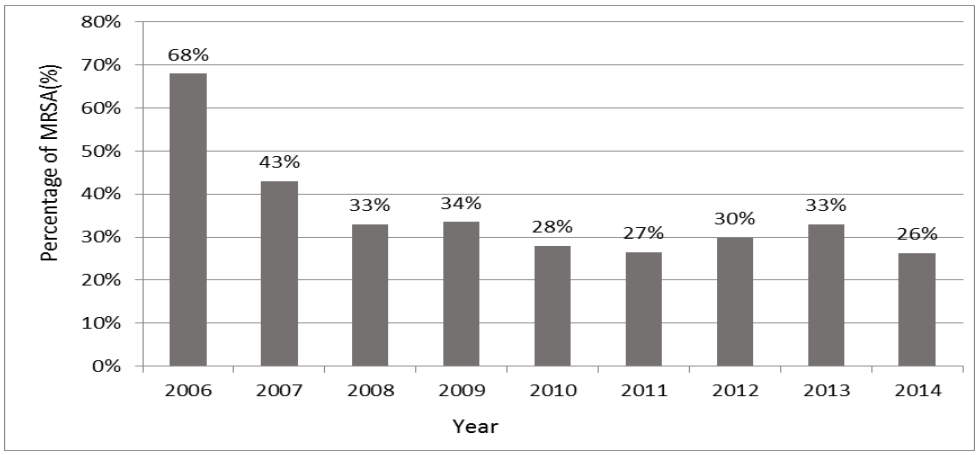

Figure.6 According to the previous report Percentage of MRSA for 9 years in the Non – ICU (All wards)

Table 1. CHROMagar MRSA Performance vs. cefoxitin disk

	Cefoxitin Disk		
MRSA Result	MRSA	Not MRSA	Total
MRSA	268	1	267
Not MRSA	13	1915	1928
	281	1916	2195
Reference Method: Cefoxitin Disk, Positive percent Agreement: 95% Negative percent Agreement: 99%			

Table 2: A comparison of the Xpert MRSA assay to rapid culture methods and conventional culture methods for the detection of methicillin –resistant *S. aureus* is shown in the Table.

Method result	%Sensitivity	% Specificity
Xpert MRSA assay	99.8%	93%
Chromogenic media	99%	93.6%
Conventional culture	100%	93.6%

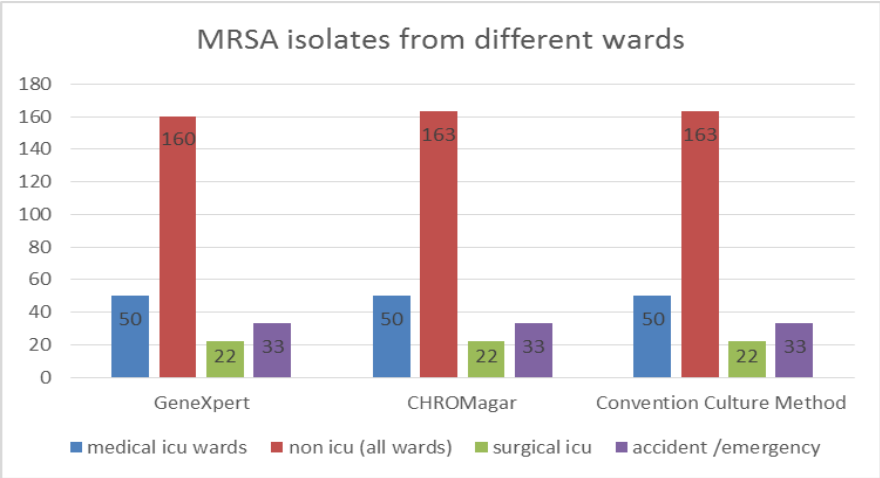

Figure 7. The overall MRSA isolates in different wards

DISCUSSION

Rapid identification of MRSA colonization is critical to the effectiveness of infection control, with delays in detection resulting in either late institution of infection control measures and resultant occult transmission of MRSA between patients or unnecessary contact precautions being applied to high-risk patient, resulting in increased hospital cost. In our study, Gene Xpert-MRSA assay and Medisinal MRSA Chromogenic media demonstrated a sensitivity and specificity above 95 % for the detection of MRSA nasal colonization compared to conventional culture method. First, Xpert MRSA is automated using the Cepheid Gene Xpert Dx System, so screening can be performed in various health care settings, such as clinical laboratory or as point-of-care testing; thus, potentially reducing the turnaround time of MRSA colonization identification. Second, the screening test method can be performed by health workers in a clinical laboratory or near the site of patient care [20].

The major promise of PCR is the lower turnaround time from admission to results reported. The BD Geneohm which we used before requires more hands –on time and are amenable to batch testing. The true value for the added speed of molecular testing is the ability to free up bed space more readily in the hospital [21]. However, [22] have demonstrated that there was a lower transmission rate in surgical patients when more rapid molecular results were implemented over culture. Currently, the nares are the only approved source of samples for MRSA screening among the FDA –approved PCR and chromogenic agents available.

The median turnaround time was less for screening using Xpert MRSA versus screening using chromogenic agar for definitive test results (1.9 h versus 66.9 h) in one study [23] and the mean overall turnaround time for Xpert MRSA was 17.1 hours compared with 53.9 h with chromogenic agar in another study [24]. The median and mean turnaround times were calculated differently in each study, which partially explain the discrepancy in the results. For instance, [24] reported a mean turnaround time of 2.6 h for screening with PCR if the time spent on specimen collection to the arrival of the specimen in the laboratory was not considered. However, the few investigations on the cost of *S. aureus* screening have focused mainly on culture-based MRSA detection and were performed at tertiary care hospitals with high MRSA rates. However, as methicillin resistance is difficult to recover from low inoculum or mixed flora samples, traditional methods are labor intensive and time-consuming and necessitate a further 2 to 3 days to confirm positives [25]. Although culture-based methods conform to the MRSA screening standard, speedier testing is of course desirable in order to resolve (or continue) precautionary infection control measures.

CONCLUSION

The impact of MRSA on hospitals remains a burden. It is imperative that an ongoing collaborative effort exists between the microbiology laboratory, infection control, pharmacy, and antibiotic stewardship program to ensure efficient infection prevention**.**

Molecular methods have the advantage of high sensitivity and rapid TATs. Rapid MRSA tests are medically reasonable tools for the timely detection of MRSA carriers, which may be particularly useful in the screening of some high-risk groups of patients. In these patients, rapid MRSA findings can be of twofold value: not only are they in the interests of patients possibly infected with MRSA (in order to start adequate treatment as early as possible), but they also serve to protect other patients from spreading the pathogen. In addition to screening, factors such as the number of contacts between healthcare workers and patients, number of patients attended by one healthcare worker per day, probability of colonization among healthcare workers, and MRSA status of hospital shared equipment and hospital environment must be considered to control the transmission of MRSA in a hospital setting. The technique must be adapted to the evolution of *S. aureus* strains, particularly for the detection of *mecA* variants isolates, the expression of which is essential for the methicillin- resistant phenotype. This would also allow for the resolution of the difficulties of interpretation of false- negative results related to *mecA* variants.

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