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ANTIMICROBIAL INVESTIGATION OF DIFFERENT MEDICATIONS AGAINST NOSOCOMIAL INFECTIONS CAUSING *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Since it was first described in the 1990s, Methicillin-Resistant *Staphylococcus aureus* infection among people with no contact with a hospital setting or with no traditional risk factors has spread worldwide and is now an important epidemiological and public health problem. This study aims to emphasize the current importance of this emergent pathogen in our area. In the present study we have analyzed the impact of modified MRSA screening of carriers and patients on epidemiological situation of MRSA during 2010-2013 in Gwalior region of Central India. All *S. aureus* isolates included in this study were identified with the use of standard microbiological methods. Susceptibility testing of all *S. aureus* isolates was done with use of the CLSI broth micro-dilution method and interpreted on the basis of CLSI criteria. The proportion of MRSA to all *S. aureus* isolates was found to be 57.6%. The plasmid carrying drug resistant gene was also reported in the biochemically identified *S. aureus* isolates. Independent of the prevention and intervention strategy in the hospitals the different MRSA incidence seems to be due to regional epidemic settings. Understanding the epidemiology of *Staphylococcus aureus* (reservoirs, transmission pathways, and risk factors) can result in excellent control of this major pathogen in hospitals and community. This finding is indicative of the spread of drug resistant *S. aureus* within health care networks, suggesting that control efforts aimed at interrupting the spread within and between health care institutions may not only be feasible but ultimately successful and should therefore be strongly encouraged.

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INTRODUCTION

Staphylococci are one of the important causes of human infections but are also found as non-pathogenic microorganisms in human samples. There is a progressive increase in MRSA prevalence and multi-drug resistance in staphylococci. Vancomycin is still the drug of choice for MRSA infections. The major reservoir of methicillin resistant staphylococci in hospitals is colonized/infected inpatients and colonized hospital workers. Resistance transfer from staphylococci to *E. coli* as well as from clinical to carrier staphylococci due to antibiotic stress seemed to be an alarming threat to antimicrobial chemotherapy. Methicillin-resistant strains of staphylococci were identified immediately upon the introduction of methicillin into clinical practice.

Methicillin-Resistant *S. aureus* (MRSA) was initially identified for the first time in 1961 by Jevons. Since then strains of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative staphylococci have spread worldwide and have become established inside and outside of the hospital environment. Already multi-resistant to different classes of antibiotics, MRSA had been reported to acquire resistance to gentamicin and related aminoglycosides therefore the treatment of infections due to these organisms and their eradication is very difficult. Constant monitoring of these strains is essential in order to control their spread in the hospital environment and transmission to the community. The present study was undertaken with the aim of determining epidemiology of clinical and carrier staphylococci and molecular studies of their acquisition and dissemination of resistance in a hospital setting in northern India.

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MATERIALS AND METHODS

The study was conducted between 2010 and 2013 on 165 human subjects, yielding a total of 356 strains of staphylococci. From clinical specimens (126), nasal cultures of hospitalized patients (143) and eye and nasal sources of hospital workers (22 and 65 respectively) strains were isolated in order to determine their epidemiology, acquisition and dissemination of resistance genes. Organisms from clinical samples were isolated, cultured and identified as per the standard routine procedures. Susceptibility was measured by the agar diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The identified bacterial pathogens were tested against 21 different antibiotics by using combi disc diffusion method (Bauer *et al.*, 1966). Reference strains included ATCC 29213 and ATCC 25923 procured from HiMedia Pvt. Ltd., Mumbai (Maharashtra), India. The modified method of Birnboim and Takahashi was used for isolation of plasmids from staphylococci.

RESULTS AND DISCUSSION

The samples collected from 165 human subjects were cultured following standard microbiological procedures. After performing gram's staining and biochemical tests for the characterization of bacterial isolates, 356 isolates were found to be Staphylococci and 232 were *Staphylococcus aureus* (coagulase positive and coagulase negative). These strains (65.2%) were then subjected to Antimicrobial susceptibility testing and 57.6% (n=134) were found to be multiple drug resistant. Among the 134 drug resistant isolates (57.6%), the most frequent antibiotic resistance for all isolates in this study were observed against Ampicillin, Oxacillin and Penicillin.

Table 1. Antibiotic resistance pattern of test bacterial organism isolated from various samples included in the study

S.No.	Antibiotics (Concentration in µg)	Resistance (%)	Sensitive (%)
1.	Amikacin (30)	25%	75%
2.	Ampicillin (10)	100%	0%
3.	Cefaperazone Sulbactam (75/30)	15%	85%
4.	Cefazolin (30)	100%	0%
5.	Cefuroxime (30)	80%	20%
6.	Cephalothin (30)	40%	60%
7.	Chloramphenicol (30)	100%	0%
8.	Ciprofloxacin (05)	83%	17%
9.	Clindamycin (02)	40%	60%
10.	Co-Trimoxazole (25)	60%	40%
11.	Erythromycin (15)	100%	0%
12.	Gentamycin (10)	100%	0%
13.	Linezolid (30)	66%	34%
14.	Ofloxacin (01)	60%	40%
15.	Oxacillin (01)	100%	0%
16.	Pazubid (25)	0%	100%
17.	Penicillin (10 units)	100%	0%
18.	Piperacillin (100)	83%	17%
19.	Teicoplanin (30)	66%	34%
20.	Tetracycline (30)	60%	40%
21.	Vancomycin (30)	10%	90%

83% isolates showed resistance against Ciprofloxacin and Piperacillin. 80% isolates were resistant to Cefuroxime. 66%

isolates showed resistance pattern against Linezolid and Teicoplanin. 60% isolates were resistant to Co-Trimoxazole, Ofloxacin and Tetracycline. 40% were resistant to Cephalothin and Clindamycin; 25% were resistant to Amikacin. Only 15% were resistant to Cefaperazone Sulbactam and 10% were resistant to Vancomycin. Pazubid25 was the only drug found to be 100% effective against all the isolates as no isolate showed resistance pattern against it. It was shown that 35.1% of *Staphylococcus aureus* and 22.5% of coagulase-negative staphylococcal isolates were resistant to oxacillin. Highest percentage of MRSA (35.5%) was found in pus specimens. In case of methicillin-resistant *Staphylococcus aureus* isolates, 9% resistance was found to vancomycin whereas highest resistance was found to penicillin G, ampicillin, cefazolin, chloramphenicol, erythromycin, oxacillin and gentamycin. It was shown that the major reservoir of methicillin resistant staphylococci in hospitals is colonized/infected inpatients and colonized hospital workers, with carriers at risk.

In the present study the results of antibiotics susceptibility testing showed that the drug resistance pattern is increasing as reported in the previous studies. Dautle *et al.*, 2002 reported that the *Staphylococcus* genus possessed the highest diversity for antibiotic resistance while organisms comprising the *Enterococcus* genus exhibited marginal levels of resistance to the antibiotics tested in their study. Approximately 43% of the isolates tested displayed multiple drug resistance, with the predominant species belonging to the *Staphylococcus* genus. In our study also test organism showed multidrug resistance. But the major concern is the development of resistance against Vancomycin which is thought to be the most effective drug against *Staphylococcus*. Among the antibiotics included in the study Ampicillin, Amikacin, Chloramphenicol, Clindamycin, Erythromycin, Gentamicin and Tetracycline are protein synthesis inhibitors. Cefaperazone Sulbactam, Cefazolin, Cefuroxime, Cephalothin, Oxacillin, Penicillin, Pazubid 25, Piperacillin and Vancomycin are cell wall inhibitor. Ciprofloxacin, Ofloxacin and Co-trimoxazole are DNA inhibitors. Activity rate of cell wall inhibitor antibiotics was reported to be better than protein synthesis inhibitors and which was more effective than DNA inhibitor antibiotics.

The present study of data gives idea about the common trend of antibiotics resistance in this region. The present study was in concordance with studies of Nagmoti *et al.*, 1999. Antibiotic resistance has increased since 2001 according to Nafessa *et al.*, 2001. Resistance pattern against Cefuroxime has been increased many folds (80%) as Saini (2003) has reported it to be 29%. Similarly, antibiotics resistance pattern has increased for all other antibiotics as well. During a period of one year, a total of 237 isolates of *S. aureus* were studied and 69 (29.1%) were found to be methicillin-resistant. MRSA isolates showed greater resistance to multiple drugs than Methicillin Sensitive *Staphylococcus aureus* (MSSA) isolates.

Inducible clindamycin resistance was 18.8% in MRSA as against 3.5% in MSSA. About 40-50% of MRSA were resistant to erythromycin, gentamicin, and chloramphenicol, while less than 30% were resistant to ciprofloxacin and Amikacin (Rao *et al.*, 2010). Sachdev *et al.* (2003) reported an outbreak of MRSA in the dermatology ward of a tertiary care hospital and described measures taken to control it. Ten patients were found to be MRSA positive over a span of three months while screening swabs from wet lesions in indoor

patients. On the basis of risk assessment, they were treated with appropriate systemic and topical therapy. All the MRSA isolates were found to be sensitive to vancomycin, teicoplanin and linezolid. Indian clinicians and infectious disease specialists in the coastal areas are facing formidable challenges from Methicillin Resistant *Staphylococcus aureus*. Despite the best surgical practices, nearly a tenth of all the SSIs cases could be caused by MRSA. Routine screening for these multidrug resistant organisms in the hospital staff, especially in the surgical departments and pre-surgical screening of the patients could help in reducing the incidence of MRSA (Naik *et al.*, 2011).

Overall study indicated that the antibiotics Cefuroxime, Ciprofloxacin, Piperacillin, Linezolid, Teicoplanin, Co-Trimoxazole, Ofloxacin, Tetracycline, Amikacin, CefaperazoneSulbactam and Vancomycin should be used judiciously against most of the staphylococcal infections in the region. Ampicillin, Cefazolin, Chloramphenicol, Erythromycin, Gentamycin, Oxacillin and Penicillin should not be used any more by general medical practitioner while giving treatment to most of the infections.

The presence of plasmid in all the multiple resistant staphylococcal isolates indicated that the resistance to multiple drugs is due to the presence of plasmid which is easily horizontally transferrable. So, such effective medications should be developed or discovered which would have counter active mechanisms against this horizontal gene transfer.

This data helps the clinicians to give proper treatment to the patient and gives idea about mostly sensitive antibiotic used in the treatment and avoid use of resistant antibiotics. A high isolation rate of pathogens from clinical specimens of clinically suspected infections showed a good correlation between clinical findings and microbiological methods. The antibiotics commonly used in such infections were less effective. Since the present study was a cross-sectional study, therefore, regular monitoring is required to establish reliable information about resistance pattern of the pathogens for empirical therapy of patients.

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