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TO DETECT LOW AND HIGH LEVEL MUPIROCIN RESISTANCE IN STAPHYLOCOCCI ISOLATED FROM VARIOUS CLINICAL SAMPLES AT SMS MEDICAL COLLEGE AND HOSPITAL, JAIPUR

Microbiology

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ABSTRACT

Background: Staphylococcal strains are the leading cause of nosocomial infections including surgical wound infections. Increasing prevalence of multi-drug resistance and low & high level mupirocin resistance among staphylococci creates a great need for regular review of antimicrobial sensitivity pattern.

Material and methods: A total of 100 staphylococci identified and antimicrobial sensitivity pattern was studied. High-level(MuH) and low-level(MuL) mupirocin resistance in Staphylococcus strains detected.

Results: Out of 100 staphylococci,57% were coagulase positive staphylococcus,43% were coagulase negative staphylococcus. Methicillin resistance detected in 56% staphylococci strains, where 37% were methicillin resistant Staphylococcus aureus and 19% methicillin resistant coagulase negative staphylococcus. MuL resistance detected in 2% of the isolates, 3.50% of total CPS and 5.40% of total MRSA.

Conclusions: Widespread use of the antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to them, hence screening for mupirocin resistance by disc diffusion method is important.

KEYWORDS

Introduction :

Staphylococcal infections are one of the most common and serious hospital as well as community acquired infections seen in developing countries[1].

Increasing resistance to a large number of antibiotics seen in cases of staphylococcal infections is a great concern for the clinicians to prevent their spread. Methicillin, an important drug of penicillin group was commonly used for these infections before the emergence of methicillin resistant *Staphylococcus aureus* (MRSA) strains[2].

S. aureus has been associated with an increased risk of infection for the colonized individual. Multidrug-resistant phenotypes are more common among methicillin resistant CoNS when compared to *S. aureus* [4].

Mupirocin (derived from *Pseudomonas fluorescens*) is an effective topical antibiotic for the elimination of methicillin resistant staphylococcus in carriers. Mupirocin (pseudomonic acid A) specifically binds to bacterial isoleucyl-tRNA synthetase (IRS) and inhibits protein synthesis. [5] Two types of mupirocin resistances are defined in staphylococci. Low-level resistance (minimum inhibitory concentrations [MICs], 8-256 µg/ml) is usually associated with point mutations in the chromosomally encoded ileS gene, whereas high-level resistance (MICs, ≥512 µg/ml) is generally due to a plasmid-mediated gene, mupA (also referred to as ileS2), which encodes an additional modified isoleucyl-tRNA synthetase.[6]

The present study was conducted to detect the antimicrobial susceptibility pattern, methicillin resistant strains and low level & high level mupirocin (MuL and MuH) resistance in staphylococcal isolates from various clinical samples obtained in the laboratory.

Material and methods:

This study comprised of total 100 samples isolated over a period of 1 year such as pus, blood, urine, respiratory samples, wound swab, and sterile body fluids from patients who attended various outpatient and inpatient departments of Sawai Man Singh Medical college and attached Hospitals, Jaipur. Samples were processed in the bacteriology section of Microbiology department.

A detailed clinical history and demographic profile was taken and recorded.

All the clinical samples were subjected to:

1. Identification of bacteria,
2. Antimicrobial susceptibility testing of isolated bacteria,
3. Detection of methicillin resistance,
4. Detection of low level and high level mupirocin (MuL and MuH) resistance.

Isolation and identification of staphylococci:

The clinical samples were processed according to standard procedures appropriate to the type of the sample for isolation and identification of bacteria.

Samples collected were inoculated on 5% Sheep Blood agar and Mac-Conkey agar, incubated at 35-37°C for 18 to 24 hours. Simultaneously specimen was inoculated in the liquid media..After incubation by gram staining, gram positive cocci in chains and clusters were identified and further subjected to various biochemical reactions as per the standard guidelines for identification.

Antimicrobial Susceptibility Testing:

Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion method. The antibiotic concentration of disc used and zone size interpretation was done in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines 2018-2019[13]

Detection of Methicillin Resistance :

Methicillin resistance was detected by using cefoxitin disc(30 µg) as per CLSI. The diameter of zone of inhibition was measured with the help of measuring scale and compared with CLSI zone size interpretative chart as

- Coagulase positive staphylococcus.
- Methicillin sensitive: if zone size was ≥22 mm
- Methicillin resistant if zone size was <21 mm
- Coagulase negative staphylococcus
- Methicillin sensitive at zone size of > 25 mm
- Methicillin resistant at zone size of <24 mm.

Detection of low level and high level mupirocin resistance :

All isolates were subjected for detection of mupirocin resistance, 5 µg for low level and 200 µg for high level as per CLSI [13]. Disc were obtained from himedia.

Interpretation of results were made on the basis of:

- Mupirocin susceptible: A zone diameter of ≥ 14 mm for both 5 µg and 200 µg disc.
- Low-level resistance: Isolates that showed zone diameters < 14 mm in the 5 µg
- disc but more than or equal to 14 mm in the 200 µg disc.
- High-level resistance: Isolates with zone diameters < 14 mm for both 5 µg and 200 µg.

S. aureus ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 were used as the control.

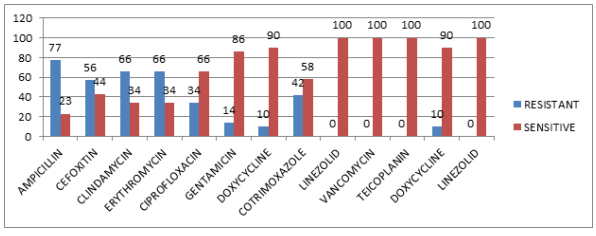
Statistical analysis:Qualitative data was expressed in terms of proportion, percentage, graphs & diagrams and analyzed by appropriate statistical tests. Qualitative data was expressed in mean & standard deviation and analyzed by appropriate statistical tests as chi square test and P value <0.05 was taken significant.

Observations and results :

Out of 100 staphylococcal isolates obtained in the present study, majority of patients were under the age of 19 years i.e. about 36% followed by 20-40 years and least number of isolates were seen in age group of 60-79 years(11%). Male to female ratio in the present study was 1.85:1. Isolates obtained from IPD (69%) were more as compared to OPD (31%).

Antimicrobial susceptibility pattern of staphylococcus isolates showed maximum resistance to ampicillin (77%), erythromycin and clindamycin(66%), cefoxitin (56%) and lower resistance to gentamicin(14%) and doxycycline(10%) as shown in figure no 1. None of the isolates showed resistance to vancomycin, teicoplanin and linezolid.

Figure no. 1: Graph shows antibiogram of staphylococcal isolates (%). (N=100)



Among 100 staphylococcal isolates, 57% were Coagulase Positive Staphylococcus (CPS) and 43% were Coagulase Negative Staphylococcus (CoNS).

Antimicrobial resistance pattern as observed in the present study for CPS and CoNS showed maximum resistance to ampicillin 84.21 % and 69.7 % respectively followed by clindamycin, erythromycin and cefoxitin.

In this study, with the cefoxitin disc methicillin resistant Staphylococcus aureus (MRSA) detected were 37%, methicillin sensitive Staphylococcus aureus (MSSA) were 20%, methicillin resistant coagulase negative staphylococcus(MRCoNS) were 19% and methicillin sensitive coagulase negative staphylococcus were (MSCoNS) were 24%.

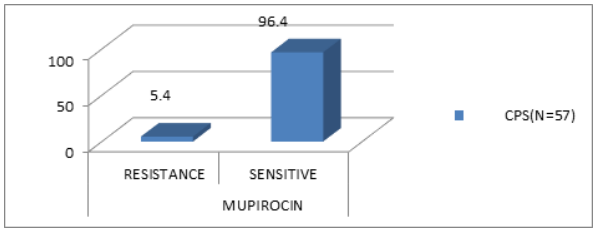
Antimicrobial resistance pattern as observed among MRSA and MRCoNS, resistance to multiple antimicrobials was more in MRSA strains as shown in table no. 1. No statistical significance was observed between the resistance pattern of MRSA and MRCoNS (P value >0.05)

Table no.1 Antimicrobial resistance pattern of MRSA and MRCoNS strains.

Antimicrobial	MRSA (n=37)		MRCoNS(n=19)	
AMPICILLIN	37	(100%)	19	(100%)
CEFOXITIN	37	100%	19	(100%)
CLINDAMYCIN	23	(62.1%)	13	(68.4%)
ERYTHROMYCIN	23	(62.1%)	14	(73.7%)
CIPROFLOXACIN	18	(48.6%)	5	(26.3%)
COTRIMOXAZOLE	18	(35.1%)	9	(47.4%)
GENTAMYCIN	6	(16.2%)	4	(21.1%)
DOXYCYCLINE	3	(8.1%)	4	(21.1%)
TEICOPLANIN	0	-	0	-
VANCOMYCIN	0	-	0	-
LINEZOLID	0	-	0	-

Out of 100 staphylococcal isolates, 2 isolates (2%) showed resistance to mupirocin and both the isolates revealed low level mupirocin resistance (MuL). Both the mupirocin resistant isolates were Coagulase Positive Staphylococci (S. aureus) (5.40%) shown in figure no.2. Another important finding was that these isolates were methicillin resistant and inducible clindamycin resistant.

Figure no.2 bar showing % mupirocin resistant CPS (N=57)



Discussion:

The present study was conducted on 100 Staphylococcus isolates obtained from various clinical samples to find out antimicrobial resistance with special reference to mupirocin resistance.

Out of 100 staphylococci , isolates obtained in the present study were more from IPD than OPD. Various other studies have also reported the same as in Kumari N et al, 2008[6] and Rudresh M.S. et al, 2015[7]. Since staphylococcus is emerging as a nosocomial infection, number of IPD is more than OPD. Age wise distribution in this study revealed that no. of staphylococci were more from samples in the age group of 0-19 years accounting for 36% followed by 20-39 years age group (32%). Some Indian study conducted by Jayakumar S. et al, 2013[8] reported about 60% isolates under age group of 40 and maximally in the age group of 21-30[9]

Sample wise distribution of isolates in this study reflected that staphylococci were maximally obtained from pus/wound swab (31%). Findings from the present study are in agreement with the other Indian studies conducted so far by Chaturvedi P et al, 2014,[2] Jayakumar S. et al, 2013[8], Shrestha B et al, 2009[13] and others. Next most common clinical sample isolated in this study after pus was blood (26%) and similar observation was seen in Chaturvedi P et al, 2014[2], AL-Zoubi M.S et al 2015[9]

Resistance pattern of isolates revealed that among all the antimicrobials tested, higher resistance was seen with β lactams, including ampicillin, followed by clindamycin and erythromycin. Similar to this study, higher resistance ranging from 65-100% % have been reported by other Indian studies. (Anupraba S et al,2003[10] , Kumari N et al,2008[6], Tiwari K et al, 2009[11], Khadri H et al,2010)[12].

In the present study, staphylococci recovered from 100 samples were 57% Coagulase Positive Staphylococcus (*Staphylococcus aureus*) and 43% Coagulase Negative Staphylococcus (CoNS). Similar results were reported from other studies conducted by Rudresh M.S. et al, 2015[7]. On the contarary to Chaturvedi P et al, 2014[2] where CoNS were than CPS.

In the present study, isolated MRSA (37%) were more as compared to MRCoNS (19%) which was very much in agreement with the other Indian studies done Rudresh, et al, 2015[7], Kaur D.C. et al,2015[5],

All the MRSA strains were more resistant to antimicrobial agents tested as compared to MRCoNS but difference in the resistance was not found to be statistically significant (p value>0.05).

In this study mupirocin resistant staphylococcal strains were 2% , out of which 3.50% were CPS and 5.40% were MRSA. Variable rate of mupirocin resistance have been reported in the studies from India and worldwide ranging from 7-15% in staphylococcal isolates and 2-17% in *Staphylococcus aureus.*

Conclusions and Summary:

Staphylococcal infection is a major concern for the medical community today. Before the time, patients commonly responded with penicillins, clindamycin, erythromycin, fluroquinolones. However with the irrationale use of these antimicrobials, staphylococci have developed resistance and emerged as MRSA causing major nosocomial morbidity[8]. Recently there is an increasing low level and high level (MuL and MuH) mupirocin resistance by the varying staphylococcal strains.

Emerging mupirocin resistance in staphylococci is a cause of serious

concern. It is recommended that routine testing of mupirocin resistance in staphylococci should be done. This will facilitate the early detection of resistance and assist in control and spread of mupirocin resistance in staphylococci.

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