

Methicillin Resistance *Staphylococcus aureus* Isolated from Surgical Site Infection in a Tertiary Care Hospital, Koshi Region (Northern Bihar), India

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ABSTRACT

BACKGROUND

Surgical site infections are the most common hospital acquired infections and are an important cause of morbidity and mortality. The objective of this study is to evaluate the causative microorganisms and antimicrobial sensitivity pattern in surgical site infections.

METHODS

A total of 300 various clinical samples received in Microbiology Department, Lord Buddha Koshi Medical College, Saharsa (Northern Bihar and Associated Hospital) from January 2019 to January 2020 were studied. In a total of 110 samples, *Staphylococcus aureus* was isolated, detected by standard biochemical methods. Antibiotic susceptibility testing was performing by Kirby Bauer Disc Diffusion method. Methicillin resistance was detected using cefoxitin (30 µg) disc diffusion method as per CLSI guidelines 2016.

RESULTS

Out of the 300 bacteria which were isolated, 160 were gram positive cocci (53.46%) and 140 were gram negative bacilli (46.54%). The most common pathogen was *Staphylococcus aureus* 109 (36.26%). The other organisms were *Escherichia*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Enterococcus*. The Antimicrobial profile of 109 *Staphylococcus aureus* isolates was as follows- among MRSA, 100% were sensitive to linezolid and vancomycin, with moderate sensitivity (72.34%) to cefuroxime, gentamicin and least sensitivity to (23.91%) doxycycline, (21.95%) ciprofloxacin.

CONCLUSIONS

The incidence of surgical site infection differs widely between surgical procedures, hospitals, patients, and between surgeons. Isolation of MRSA patients and carriers in the hospitals, regular observation, and monitoring of antibiotic susceptibility pattern of the hospital and community of that region frequently and formulation of antibiotic policy may help in reducing the treatment failures.

KEYWORDS

Methicillin Resistant *Staphylococcus aureus*, Surgical Site Infections (SSI), Antimicrobial Sensitivity

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BACKGROUND

Surgical Site Infections (SSI) are caused by the exogenous and endogenous microorganisms that enter the operative wound during the course of the surgery.¹ The frequency of surgical site infection differs widely between surgical procedures, hospitals, patients and between surgeons.² The most commonly isolated microorganisms are *Staphylococcus aureus* (*S. aureus*), Streptococci, Enterococci, *E. coli*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Acinetobacter*, *Proteus*, etc. *S. aureus* is the commonest cause of SSI and other nosocomial infections. *S. aureus* was once susceptible to Penicillin, but widely resistant organisms soon emerged. The introduction of Methicillin initially solved the problem, but later, strains which were resistant to Methicillin developed. Thus, an increased number of resistant strains have been seen worldwide.^{3,4}

Methicillin-resistant *Staphylococcus aureus* (MRSA) is heightened by the fact that these isolates are usually resistant to other anti-staphylococcal agents (clindamycin, Erythromycin, Tetracycline, sometimes Gentamicin and Trimethoprim/ Sulphamethoxazole), with the exception of Vancomycin. Sometimes, Methicillin-resistant-*Staphylococcus aureus* appears to be susceptible in vitro to other β -lactam agents such as Cephalosporins; however, they are clinically ineffective.⁴ The incidence of MRSA in SSI is increasing more in developing countries because of lack of general hygienic. The present study was undertaken to determine the bacteriological profile and antibiogram of surgical site infections in Lord Buddha Koshi Medical College, Saharsa.

METHODS

Study Population

This study was conducted in the Department of Microbiology, Department of Pharmacology, Lord Buddha Koshi Medical College, Saharsa Bihar and Associated Hospital. From January 2019 to January 2020. The study population included three hundred patients suffering from surgical site infections in the Obstetrics and Gynaecology department of Lord Buddha Koshi Medical College, Saharsa, Bihar and Associated Hospital. These patients were selected randomly and they belonged to the age group of five to sixty five years.

Inclusion Criteria

A surgical wound with pus discharge, wounds discharge and negative cultures, warmth, erythema, induration and pain and the physician diagnosis were considered as surgical site infection.^{5,6}

Exclusion Criteria

Wounds with cellulitis and no drainage and suture abscesses were not included in the study.

Specimen Collection

Clinical history regarding the age, sex, type of illness, diagnosis, the type of operation performed, antibiotics given and the presence of associated diseases like diabetes and peripheral vascular disease was obtained. Pus samples were collected from each patient with the help of two sterile swabs used for smear preparation and the other was used for culture.

Specimen Transport

The swabs were brought to the Department of Microbiology, Lord Buddha Koshi Medical College, Saharsa, Bihar and Associated Hospital Bihar, immediately and processed within thirty minutes of collection.

Sample Processing

The pus samples were inoculated onto the media immediately and were incubated at 37°C for 24 hours of incubation, the isolated organisms were identified by standard methods.^{5,6} Preliminary identification of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done by the disc diffusion test which was described by the modified Kirby Bauer method. The drugs were chosen, based on their action on a particular organism and also on the antibiotic policy of the hospitals. Six discs were used on a 9 cm diameter plate. The antimicrobial discs for *Staphylococcus aureus* were: Penicillin (10 units), Erythromycin (15 mcg), Ciprofloxacin (5 mcg), Cefoperazone (30 mcg), Oxacillin (1 mcg), and Co-trimoxazole (25 mcg). These were tested as first line antibiotics. If zone diameter was ≥ 13 mm, it was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA) and if it was ≤ 10 mm then it was considered as MRSA. *S. aureus* ATCC 25923 was used as a control strain for the standardization of antimicrobial susceptibility testing.

Statistical Analysis

Statistically analysed using SPSS Data Editor Software, Chicago, and version 20. The statistical methicillin resistant *S. aureus* isolates were evaluated using Chi-square test and $p < 0.05$ was considered as statistically significant.

RESULTS

Out of the 300 bacteria which were isolated, 160 were gram positive cocci (53.46%) and 140 were gram negative bacilli (46.54%). The most common pathogen followed by *Staphylococcus aureus* 109 (36.33%). Extra organisms were *Escherichia*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Enterococcus*.

Aerobic Organisms		No. of Isolates	%
Gram Positive Cocci	<i>Staphylococcus aureus</i>	109	(36.33%)
	<i>Staphylococcus epidermidis</i>	40	13.33
	Group A beta haemolytic <i>Streptococcus</i>	7	2.33
	<i>Enterococcus faecalis</i>	4	1.33
	Total	160	53.46
Gram Negative Bacilli	<i>Pseudomonas</i> species	55	18.33
	<i>Escherichia coli</i>	27	9.00
	<i>Acinetobacter</i> species	19	6.33
	<i>Klebsiella</i> species	15	5.00
	<i>Proteus</i> species	13	4.33
	<i>Citrobacter</i> species	7	2.33
	<i>Enterobacter</i> species	4	1.33
Total	140	46.66	

Table 1. Bacteria Isolated from Infected Postoperative Wounds
Chi-square test: X²= 182.30; P value= <.05 statistically significant

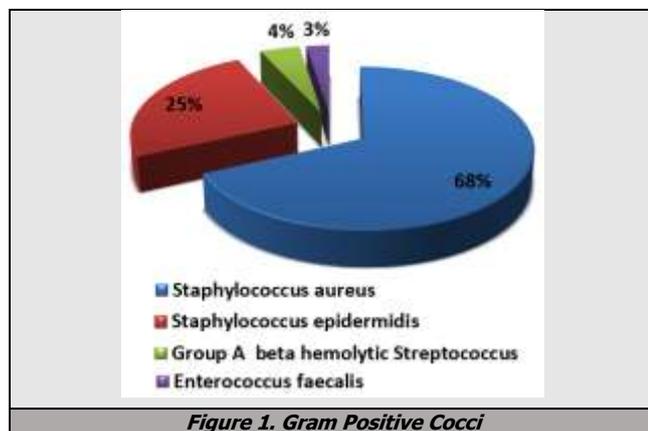


Figure 1. Gram Positive Cocci

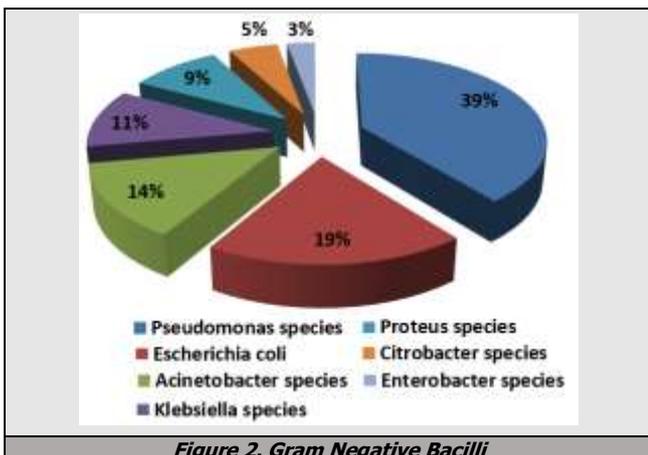


Figure 2. Gram Negative Bacilli

Types of Sample	Samples No.	Samples %
Pus	59	54.12%
Blood	27	24.77%
Urine	6	05.50%
Sputum	8	07.33%
Miscellaneous*	9	08.25%
Total	109	100

Table 2. Sample-Wise Distribution of S. aureus Isolates (n=109)

(Note*: - Miscellaneous samples include ear discharge, abdominal drain fluid, throat swab, conjunctival swab and wound discharges etc.)

Antibiotic Tested	Total No. N = 109 (100%)	
	MARSA No. (%)	MSSA No. (%)
Vancomycin	0 (00.00%)	109 (100%)
Linezolid	0 (00.00%)	109 (100%)
Ciprofloxacin	80 (78.05%)	21 (21.95%)
Cefoxitin	25 (24.76%)	75 (75.24%)
Gentamicin	23 (27.66%)	71 (72.34%)
Cefuroxime	23 (27.66%)	71 (72.34%)
Amoxiclav	36 (36.19%)	64 (63.81%)
Amoxicillin	1 (32.33%)	1 (67.77%)
Doxycycline	76 (76.09%)	24 (23.91%)
Levofloxacin	40 (40%)	60 (60%)

Table 3. Resistance to Individual Antimicrobials in MRSA and MSSA Isolated in Saharsa

N = Number of Isolates

Among 109 *S. aureus* isolates included in our study, 59 (54.12%) were isolated from pus samples, 27 (24.77%) were isolated from blood, 6 (5.50%) were isolated from Urine, 8 (7.33%) were isolated from sputum, and 9 (8.25%) were isolated from miscellaneous samples as shown in (Table 2). Out of 109 *S. aureus* isolates, 37 (36.6%) were methicillin resistant (MRSA) and 64 (63.4%) were methicillin-sensitive *S. aureus* (MSSA). Out of 109 *S. aureus* isolates of the MRSA 37 (36.5%) were derived from respectively Pus samples 15 (41.09%). The Antimicrobial profile of 109 *Staphylococcus aureus* isolates among MRSA, resistance those they were 100% sensitive to linezolid and vancomycin, with moderate sensitivity (72.34%) to cefuroxime, gentamicin and least sensitivity to (23.91%) doxycycline, (21.95%) ciprofloxacin. (Table 3).

DISCUSSION

The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital showed a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008.⁷ A recent study⁸ found the prevalence to be 42% in 2008 and 40% in 2009. In a study at Aligarh, India⁹ it was shown that 35.1% of *S. aureus* and 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. In another study¹⁰ conducted in Tamilnadu, out of 906 strains of *S. aureus* isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. Our study had MRSA prevalence of 36.5%. This variation in prevalence may be because of several factors like healthcare facilities available in the particular hospital, implementation and monitoring of infection control committee, rationale antibiotic usage which varies from hospital to hospital.

In our study, we have included 109 *S. aureus* isolates derived from pus 59 (54.12%), blood samples were 27 (24.77%), Miscellaneous Samples were 09 (08.25%) and urine sample 6 (5.50%) from both outpatients and inpatients of Microbiology Department of our Institution. The prevalence of Methicillin resistance amongst all *S. aureus* isolates was found to be 36.5%. This difference could be due to prolonged hospital stay, instrumentation and other invasive procedures. A comparable prevalence rate of 24, 34.6%, and 36.6% were also reported from Northern Bihar, and West Champaran Bihar.^{11,12,13}

Although MRSA from clinical specimens showed higher susceptibility to individual antibiotics when compared with others, we obtained high percentage of multidrug resistant MRSA from these specimens. Poddar CK, et al. From Bihar had reported 24% of the MRSA isolated from clinical specimens to be multidrug resistant.¹¹ Pappu RK, Poddar CK, et al. From Northern Bihar had reported a higher percentage of multidrug resistant MRSA.¹² Rajak KC, Poddar CK, et al. From Bihar reported even a higher percentage of multidrug MRSA but from high risk patients admitted in burns and orthopaedic units.¹³

In our study we also looked forward for treatment options for Methicillin resistant *S. aureus* isolates by

detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with Methicillin resistant *S. aureus* isolates were 100% susceptible to linezolid and vancomycin, followed by moderate susceptibility (72,34%) to gentamicin, cefuroxime and least susceptibility to doxycycline, ciprofloxacin (23.91% and 21.95% respectively). This finding is in concordance to other studies that also found that all the Methicillin resistant *S. aureus* isolates were uniformly susceptible to linezolid and vancomycin.^{14,15,16}

CONCLUSIONS

The degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse depending on the region and vancomycin was the only antibiotic found to give uniform sensitivity (100%). When antimicrobials including vancomycin are considered for treatment, choice inevitably requires the need for in vitro susceptibility testing of every isolate of MRSA in the clinical laboratories. Our study is a preamble to enable epidemiologists to understand the nature of MRSA isolates in Saharsa, Bihar, India.

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