Antimicrobial Susceptibility of Organisms Isolated from Surgical Site Infection in a Tertiary Care Hospital, Bettiah (West Champaran) Bihar, India

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ABSTRACT

Introduction: Surgical site infections are most common hospitals acquired infections and are an important cause of morbidity and mortality. The objective of our study is to conclude the causative bacteria and antimicrobial sensitivity of surgical site infections.

Material and methods: A total of 275 various clinical samples received in Microbiology Laboratory, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. from March 2018 to April 2019. A total 101 Staphylococcus aureus isolated, were identified by standard biochemical methods. Antibiotic susceptibility testing was performed by Kirby Bauer Disc Diffusion method. Methicillin resistance was detected by using cefoxitin (30µg) disc diffusion method as per CLSI guidelines 2016.

Result: Out of the 275 aerobic bacteria which were isolated, 144 were gram positive cocci (52.37%) and 131 were gram negative bacilli (47.63%). The most common pathogen followed by Staphylococcus aureus 101 (36.36%). Other organisms were Escherichia, Pseudomonas, Klebsiella, Citrobacter, Proteus, and Enterococcus. The Antimicrobial profile of 101 Staphylococcus aureus isolates among MRSA, resistance those they were 100% sensitive to linezolid and vancomycin, with moderate sensitivity (71.14%) to cefuroxime, gentamicin and least sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin.

Conclusion: Isolation of MRSA patients and carriers in the hospitals, regular surveillance, and monitoring of antibiotic susceptibility pattern of the hospital and community of that region regularly and formulation of antibiotic policy may help in reducing the treatment failures.

Keywords: Surgical Site Infections (SSI), Methicillin Resistant Staphylococcus Aureus, Antimicrobial Sensitivity.

INTRODUCTION

Surgical site infections (SSI), one of the most common causes of healthcare associated infections are a common complication associated with surgery, with a reported incidence rates of 2-20%.[1] Surgical site infections (SSI) are a vital cause by the exogenous and endogenous microorganisms that enter the operative wound during the surgery.[2] The incidence of surgical site infection differs widely between surgical procedures, hospitals, patients and between surgeons.[3]

The most frequently isolated bacterial pathogens are Staphylococcus aureus (S.aureus), Streptococci, Enterococci, E.coli, Klebsiella, Enterobacter, Citrobacter, Acinetobacter, Proteus, etc. S.aureus form a part of the normal flora and can be isolated from the noses of up to 60% of the healthy individuals. It is readily transmitted from individual to individual onto the hands and clothes of the health care staff, onto objects and into the air.[2,4] S.aureus is the common reason of SSI and other nosocomial infections. S.aureus was once susceptible to Penicillin but largely resistant organisms soon emerged. The introduction of Methicillin primarily solves the problem, but later, the strains which were resistant to Methicillin developed. Thus, an increased number of resistant strains have been seen internationally.[4,5]

The clinical consequence of Methicillin-resistant Staphylococcus Aureus (MRSA) is delicate by the fact that these isolates are frequently resistant to other anti-staphylococcal agents (Clindamycin, Erythromycin, Tetracycline, sometimes Gentamicin and Trimethoprim/ Sulphomethoxazole), with the exception of Vancomycin. Sometimes, Methicillin-resistant-Staphylococcus aureus appear to be susceptible in vitro to other β-lactam agents such as Cephalosporins; however, they are clinically unsuccessful.[5] Since MRSAs are resistant to all the β-lactam antibiotics, the therapeutic options are extensively limited. The incidence of MRSA in India range from 30-70%.[6,7]

The prevalence of MRSA in SSI is increasing more in developing countries because of lack of general hygiene. The present study was undertaken to establish the bacteriological

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profile and antibiogram of surgical site infections.

MATERIAL AND METHODS

This study was conduct in the Department of Microbiology, Department of Pharmacology, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. from March 2018 to April 2019. The study population included two hundred seventy five patients suffering from surgical site infections in the surgical and orthopaedic departments of Government Medical College, Bettiah (West Champaran), the Obstetrics and Gynaecology department of Government Medical College, Bettiah (West Champaran) 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 site infection with wound discharge, pus discharge and negative cultures, but with symbols of sepsis present concurrently (warmth, erythema, induration and pain) and the physician diagnosis was considered as surgical site infection.[9]

Exclusion criteria

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

Relevant history

A small clinical history concerning the age, sex, type of illness, diagnosis, the type of procedure achieves, antibiotics given and the incidence of related diseases like diabetes and peripheral vascular disease was obtained.

Specimen collection

Pus samples were assembling 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, Department of Pharmacology, Government Medical College, Bettiah (West Champaran) 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 in 7-10% CO2 concentration. After 24 hours of incubation, the isolated organisms were recognized by standard methods.[8,9] Preliminary detection of bacteria was based on colony characteristics of the organisms. Such as haemolysis on blood agar, changes in physical appearance. Gram-negative rods were recognized by performing a sequence of biochemical tests. Namely: Indole, urea, Simon's citrate agar and motility. Gram-positive cocci were recognized based on their gram reaction, catalase and coagulase test results.

Antimicrobial susceptibility testing[10,11]

Antibiotic susceptibility testing was used the disc diffusion test which described by the Kirby Bauer method. The antimicrobial containing discs was located on the agar plate within 15 minutes of inoculation by using sterile forceps and these were pressed firmly beside the plate. The plates were inverted and incubated for 18-24 hours at 35°C, at a CO2 concentration of 7-10%.[12] The drugs were selected, based on the antibiotic guidelines of our hospitals. Six discs were used on a 9 cm diameter plate. The antimicrobial discs for Staphylococcus aureus were: Penicillin (10 units), Ciprofloxacin (5mcg), Erythromycin (15mcg), Cefoperazone (30mcg), Oxacillin (l mcg) and Co-trimoxazole (25mcg). These were identified as first line antibiotics. Those which were resistant to the first line antibiotics were identified with second line drugs like Vancomycin (30mcg), Rifampicin (30mcg), Teicoplanin (30mcg),. Cephotaxime (30mcg) and Amoxyclav(30mcg). The antibiotic discs obtained from HiMedia Laboratories, Pvt. Ltd. Mumbai. After 18-24 hours of incubation, the diameter of the inhibitory zone was measured by using a millimeter scale. The zone size around each antimicrobial disc was interpreted MRSA recognition was done using oxacillin disc (1 µg) and Mueller Hinton agar with 2% NaCl. The plates were incubated for 24 hours at 35˚C and zone diameter was measured. If zone diameter was ≥13mm, it was measured as Methicillin sensitive Staphylococcus aureus (MSSA) and if it was ≤10 mm then it was measured as MRSA.[12] All MRSA and MSSA strains were identified for their susceptibility to all antibiotics include ciprofloxacin, co-trimoxazole, amikacin, clindamycin, erythromycin, chloramphenicol, cephalexin, gentamicin, vancomycin, penicillin, linezolid, amoxicillin, amoxyclav, cefuroxime, and cefotaxime by Kirby-Bauer disc diffusion technique.[12] All tests were perform on Mueller-Hinton agar and were interpret after 24 hours of incubation at 35˚C. The inhibition zone diameters were measured around each disc and were interpret according to the Clinical Laboratory Standards Institute guidelines.[12] S. aureus ATCC 25923 was used as antimicrobial susceptibility testing.

STATISTICAL ANALYSIS

The collected data were statistically analyzed using SPSS Software. The statistical methicillin resistant S. aureus isolate were evaluated using Chi-square test and $p < 0.05$ was measured as statistically significant.

RESULT

Out of the 275 aerobic bacteria which were isolated (Table 1/Fig-1, 2), 144 were gram positive cocci (52.37%) and 131 were gram negative bacilli (47.63%). The most common organism was Staphylococcus aureus, with 101 isolates (36.36%). Other predominant Pseudomonas, Escherichia, Citrobacter, Klebsiella, Proteus, and Enterococcus.

Among 101 S. aureus isolates included in our study, 57 (57%) were isolated from pus samples, 26 (25.70%) were isolated from blood, 5 (5.2%) were isolated from Urine, 5 (4.8%) were isolated from sputum, and 8 (7.3%) were isolated from miscellaneous samples as exposed in [Table 2].

Out of 101 S. aureus isolates, 37 (36.6%) were methicillin resistant S. aureus (MRSA) and 64 (63.4%) were methicillin-sensitive S. aureus (MSSA). while, the preponderance of

Aerobic Organisms	No. of isolates	Percentage	
Gram Positive Cocci	Staphylococcus aureus	101	36.66
	Staphylococcus epidermidis	35	12.77
	Group A beta hemolytic Streptococcus	5	1.66
	Enterococcus faecalis	3	1.11
	Total	144	52.2
Gram Negative Bacilli	Pseudomonas species	53	19.45
	Escherichia coli	26	9.45
	Acinetobacter species	17	6.12
	Klebsiella species	14	5.00
	Proteus species	12	4.45
	Citrobacter species	6	2.22
	Enterobacter species	3	1,11
	Total	131	47.8

Table-1: Aerobic bacteria isolated from infected postoperative wounds Chi-square test: X2= 182.30; P value= <.05 statistically significant.

Types of Sample	Samples Number	Samples Percentage
Pus	57	57.00%
Blood	26	25.70%
Urine	5	05.20%
Sputum	5	04.80%
Miscellaneous*	8	07.30%
Total	101	100

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

Table-2: Sample-wise distribution of S.aureus isolates [n=101]

Sample	Staphylococcus aureus			Chi-Square ($\chi 2$) & *p value $X^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{i,j}-E_{i,j})^2}{E_{i,j}}$.
	Resistant to cefoxitin (MRSA) N = 37 (36.5%)	Susceptibile to cefoxitin (MSSA) N = 64 (63.5%)	Total isolates N = 101 (100%)	
Pus due to any other cause, N(%)	15 (34.23%)	30 (65.77%)	45(44.4%)	Chi^2=9.6316 DF=4; P=0.047113 statistically significant
Post operative Wound infection, N (%)	7 (52.38%)	6 (47.62%)	13(12.6%)	
Blood; and SNCU blood culture, N (%)	9 (31.78%)	18 (68.22%)	25(25.7%)	
Miscellaneous* Sample, N (%)	5 (41.67%)	7 (58.33%)	13(12.1%)	
Urine due to any other cause, N (%)	02(36.36%)	04(63.63%)	06(05.2%)	

N = Number of isolates; MSSA = Methicillin sensitive Staphylococcus aureus; MRSA = Methicillin resistant Staphylococcus aureus; *p value < 0.05 was considered as statistically significant.

Table-3: Distribution of Staphylococcus aureus isolates on the basis of sample and susceptibility to cefoxitin (30 µg) disk.

Antibiotic tested	Total No. N = 101 (100%)	
	MARSA NO. (%)	MSSA NO. (%)
Vancomycin	0(00.00%)	101(100%)
Linezolid	0(00.00%)	101(100%)
Ciprofloxacin	80 (79.05%)	21(20.95%)
Cefoxitin	25(24.76%)	75 (75.24%)
Gentamicin	23(22.86%)	71(71.14%)
Cefuroxime	23(22.86%)	71(71.14%)
Amoxyclav	36 (36.19%)	64(63.81%)
Amoxicillin	1(33.33%)	1(67.77%)
Doxycycline	76 (76.19%)	24 (23.81%)
Levofloxacin	40(40%)	60(60%)

N = Number of isolates

Table-4: Resistance to individual antimicrobials in MRSA and MSSA isolated in Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. Bihar.

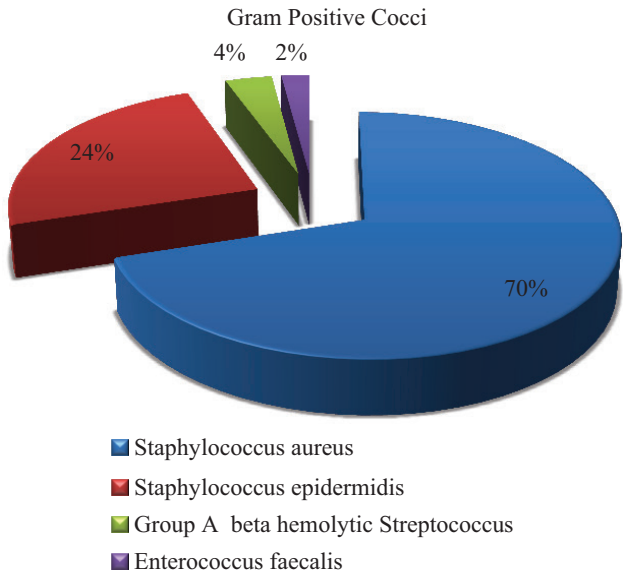

Figure-1: Gram positive cocci

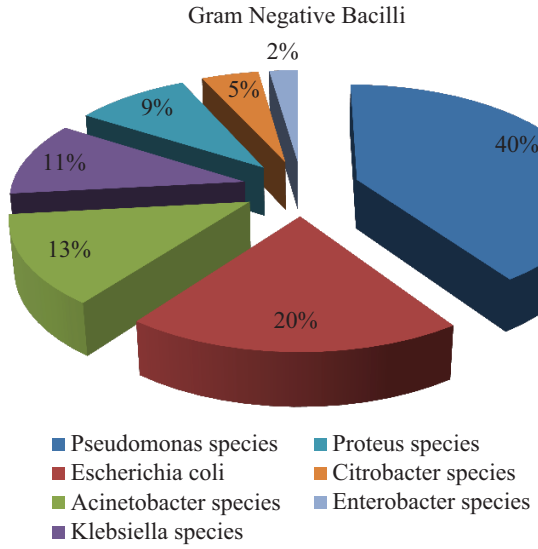

Figure-2: Gram negative bacilli

the MRSA isolates were consequent from pus samples 15, however, the S. aureus isolates consequent from post-operative wound infection were mostly MRSA 7. This finding was found to be statistically significant (P=0.008904). [Table-3]

Out of 101 S. aureus isolates of the MRSA 37 (36.5%) were derived from respectively Pus samples 15 (41.09%), the S. aureus isolates derived from Wound Samples were MRSA 7 (17.80%), the S. aureus isolates derived from Blood Samples were MRSA 8 (21.91%), the S. aureus isolates resulted from Miscellaneous Samples were MRSA 05 (13.69%) and the S. aureus isolates resulted from Urine Samples were MRSA 2 (5.47%). This finding was found to be statistically significant (p=0.347486).[Table-3]

The antimicrobial susceptibility test result of all the 101 S. aureus isolates Among MRSA, resistance that they were 100% sensitive to vancomycin and linezolid, with moderate sensitivity (71.14%) to gentamicin, cefuroxime and least sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin as shown in [Table 4].

DISCUSSION

There is a growing concern in India, the importance of MRSA as a problem has been recognized comparatively late.[13] The prevalence of MRSA varies in different parts of India and is not uniform. Reports from a Delhi hospital show a prevalence rate of 51.6% in 2001, whereas it was reported as 38.44% in the same hospital in 2008.[14]

A recent study[15] found the predominance to be 42% in 2008 and 40% in 2009. In a study at Aligarh, India[16] it was shown that 35.1% of S. aureus and 22.5% of coagulates-negative staphylococcal isolates were resistant to methicillin. In another study[13] 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 predominance 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 101 S. aureus isolates derived from pus 45 (44.40%), post-operative wound infection 13 (12.60%), blood samples were 25 (25.70%), Miscellaneous Samples were 13 (12.10%) and urine sample 6 (5.20%) from both outpatients and inpatients of Orthopaedic 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.[17,18,19]

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 from Bihar had reported 24% of the MRSA isolated from clinical specimens to be multidrug resistant.[17] Northern Bihar had reported a higher percentage of multidrug resistant MRSA.[18] Bihar reported even a higher percentage of multidrug MRSA but from high risk patients admitted in burns and orthopedic units.[19]

In our study we also looked forward for 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 (71,14%) to gentamicin, cefuroxime and least susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% 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.[20,21,22]

CONCLUSION

In conclusion, the degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to 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 Bettiah (West Champaran) Bihar, India.

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