Int.J.Curr.Microbiol.App.Sci (2021) *10*(11): 369-375

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 11 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1011.042

Antimicrobial Susceptibility Pattern of Bacterial Isolates from Surgical Site Infections in a Tertiary Care Hospital

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Keywords

Surgical site infections (SSI), methicillin resistant *Staphylococcus aureus* (MRSA)

Article Info

Received:
14 October 2021
Accepted:
04 November 2021
Available Online:
10 November 2021

A B S T R A C T

Background Surgical site infections are ranked among the most common health care associated infections. They cause significant morbidity, increased cost of care and prolonged hospital stay. A spectrum of microorganisms with varied antimicrobial susceptibility patterns have been identified as causative agents of SSI which vary with time, hospital, and with the type of surgical procedure performed. We conducted this study with an objective to assess the burden of SSI, its causative aerobic bacteria and their *in vitro* antibiotic susceptibility patterns. Aims & objectives 1) To identify the aerobic bacteriological profile of isolates causing surgical site infections. 2) To determine the antibiotic sensitivity pattern of the isolates. Method: This study includes 266 clinically diagnosed cases of SSIs over a period of 8 months. Isolates were identified by conventional methods. Isolates of *Staphylococcus aureus* were tested for methicillin resistance by cefoxitin. Isolates of Escherichia coli and *Klebsiella pneumoniae* which showed resistance to cefotaxime and ceftazidime were tested for ESBL production by CLSI guidelines. Isolates of *Pseudomonas aeruginosa* were screened for MBL production using Imipenem disc diffusion test. Results of the 266 samples processed, 193(72.5%) were culture positive samples which yielded 204 isolates. *Staphylococcus aureus* 60 (29.4%) was found to be the predominant organism causing SSI followed by *Pseudomonas aeruginosa* 40 (19.6%). Methicillin resistance was observed in 12 (20%) of *Staphylococcus aureus* strains. ESBL production was observed in 20.5% of *Klebsiella pneumoniae* isolates and 13.2 % *Escherichia coli* isolates. MBL production was not seen *Pseudomonas aeruginosa* isolates. Conclusion: The present study showed the commonest bacteria responsible for the surgical site infections like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Hence Implementation of an effective infection control programme and judicious use of antibiotic prophylaxis reduces the incidence of SSI in the hospital.

Introduction

Surgical site infections (SSI) are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections. Most nosocomial surgical site infections (60-80%) occur in the incision, some involve deep soft tissue or adjacent sites. [1]Several studies have demonstrated a prolonged stay of hospitalization and associated financial burdens for patients with SSI compared to non-infected patients underwent similar surgical procedures.[2,3] The risk of developing SSI is affected by many factors. These include the degree of microbial contamination of the operation site indicated by wound class as clean, clean contaminated, contaminated and dirty, and also by patient age, length of surgery, pre-operative shaving of the operative site, hypothermia and co-morbidities e.g. diabetes and obesity.[4]

The accumulation of serous, serosanguinous or purulent material either as abscess or exuding from a muco-cutaneous surface is one of the signs of local infection.[5] Sources of surgical site infections can include the patient's own normal flora or organisms present in the hospital environment. The common organisms encountered in post-operative wound infections are *Staphylococcus aureus, CONS, Enterococci, Proteus, Pseudomonas, Escherichia coli* and *Klebsiella species*.[6,7] This study is being undertaken to identify the common causative organisms of surgical site infections and suggest effective antibiotics which will help to reduce morbidity, mortality and duration of stay in the hospital.

Materials and Methods

This study was carried out in the department of Microbiology, Dr. Chandramma Dayananda Sagar institute of Medical education and research, Harohalli, Ramanagara Dt, Karnataka during the period of 8 months from January2021 to August2021. Samples were collected after obtaining consent. Institutional ethics committee approval was obtained. Inclusion criteria:

All clinically diagnosed cases of surgical site infections of all age groups, irrespective of preoperative administration of antibiotics,

Those who stayed for at least seven days post-operatively were included in this study.

Exclusion criteria

Cases of anaerobic bacteria and fungal isolates were excluded from the study.

Sample collection and method

A total of 266 clinically diagnosed cases of surgical site infections admitted in various surgical wards at Dr. Chandramma Dayananda Sagar institute of Medical education and research, Harohalli, Ramanagara Dt, Karnataka were included in this study. Samples were received in laboratory either as a wound swab or as an aspirate from the infected wound. The samples were immediately cultured on bloodagar, MacConkey's agar. Bacterial isolates were identified as per standard protocol.[8]

Antimicrobial susceptibility testing

This was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method according to the CLSI guidelines 2018.[9] Bacterial isolates were tested against ampicillin/sulbactum (10µg/10µg), amikacin (30µg), gentamicin (10µg), cefotaxime (30µg), ceftriaxone (30µg), ceftazidime (30µg), cotrimoxazole (1.25/23.75µg), ciprofloxacin (10µg), Piperacillin(100µg) piperacillin-tazobactum (100/10µg),

cefeperazone-sulbactum (30µg/10µg), cefepime (30µg), Imipenem (10µg). Staphylococci were tested against Amoxy-clav (10µg/10µg), amikacin (30µg), gentamicin (10µg) high-level gentamicin (120µg) for enterococci), ceftriaxone (30µg), ciprofloxacin (10µg), erythromycin (15µg), clindamycin (2µg), cotrimoxazole (1.25/23.75µg), vancomycin (30µg), linezolid (30µg) and cefoxitin (30µg).

Test for Detection of ESBL Production in Enterobacteriaceae

Isolates which were resistant to third generation cephalosporins were tested for ESBL production by combination disk method using cefotaxime (30µg), cefotaxime/ clavulanic acid (30 µg/10µg), and ceftazidime (30µg), ceftazidime/ clavulanic acid (30µg/10µg). Plates were incubated overnight at 37˚C. Zone of inhibition of ≥5 mm around cephalosporin + clavulanate compared to cephalosporin alone confirms ESBL production.[9]

Test for detection of Methicillin resistance in Staphylococcus

The test was carried out on Mueller-Hinton agar using a cefoxitin disc (30µg) and incubated at 35˚C for 18-24 hrs. An inhibition zone diameter of ≤ 21mm was reported as methicillin resistant and a diameter of ≥ 22 mm was reported as methicillin sensitive strains.[9]

Results and Discussion

A total number of 266 samples were collected during the period of study, out of which 193(72.5%) were culture positive samples which yielded 204 isolates. Most of the samples were obtained from individuals between the age of 40-60 yrs. Infection rate was more common in males(66.3%) compared to females(36.7%). The most common isolate were *Staphylococcus aureus* 60 (29.4%), *Pseudomonas aeruginosa* 40(19.6%), *Klebsiella pneumoniae* 34(16.6%), *Escherichia coli* 29 (14.2%) followed by *Staphylococcus epidermidis* 16 (7.8%) *Proteus mirabilis* 11 (5.3%), *Citrobacter koseri* 8 (3.9%), *Enterococcus* species 4 (1.9%), *Acinetobacter* species 2 (0.9%) (Table1)

The predominant isolate *Staphylococcus aureus* 60 (29.4%) showed maximum sensitivity towards vancomycin (100%), linezolid (100%), amikacin (96%), Gentamycin (89.7%) and least sensitive to ciprofloxacin (22.2%), amoxyclav (33.3%), ceftriaxone (52.3%).(Table 2)

Among the 60(29.4%) *Staphylococcus aureus* isolates, 12 (20%) were found to be MRSA by cefoxitin disc diffusion test. All the MRSA strains were found to be 100% sensitive to Vancomycin and Linezolid.(Table 4)

Pseudomonas aeruginosa 40(19.6%) was the second most common isolated organism and it was most sensitive to Imipenem(100%), Piperacillin-tazobactum (85%), and least sensitive to Piperacillin (14.2%), cefepime (37.5%), ciprofloxacin (47.5%).

Klebsiella pneumoniae 34(16.6%)was the third most common isolated organism and it was most sensitivite to Imipenem (100%), piperacillin-tazobactum (91.2%) amikacin (60%) and least sensitive to ampicillin/sulbactum (21%), cotrimoxazole (45.6%) and ciprofloxacin (54.3 %).

*Escherichia coli*29(14.2%) was found to be maximum sensitivity towards Imipenem (100%), piperacillin-tazobactum (94.5%), Amikacin (64.2%) and they were least sensitive towards ampicillin/sulbactum (21.6%), ciprofloxacin (37.5%) and

cotrimoxazole (52%).(Table 3). Among the 68 Enterobacteriaceae isolates, 23 (33.8%) were ESBL producers. Highest prevalence of ESBL production was seen in *Klebsiella pneumoniae* 14 (20.5%), followed by *Escherichia coli* 9(13.2%). The ESBL producing strains showed maximum sensitivity towards Imipenem and piperacillin-tazobactum and 100% resistance towards third generation cephalosporins and ampicillin/sulbactum.

Surgical site infections still remains one of the most important causes of morbidity and is the most common nosocomial infection in surgically treated patients.[2,10] The problem of post operative wound infection is seen in both developing and developed countries despite meticulous surgical practice and proper sterilization techniques.[11]

The incidence of SSI were the most common health-care associated infection accounting for 31% of all HAI among hospitalized patients.[12]

In our study incidence of SSI was higher in males (66.3%) as compared to females (36.7%). Male gender is associated with a dramatically increased risk of major infections following trauma. A study done by kumar *et al.*, showed a similar trend 25.6 % in male and 17.4 % in female.[13]

Rate of SSI increase with the increase in age. In the present study a higher proportion of SSI was found above the age of 50 yrs. This is similar to other studies.[14-1] This is due to poor immune response, underlying risk factors in old age, and reduced compliance with treatment.[6] In our study, we obtained 193 culture positive samples which yielded 204 isolates from 266 suspected postoperative wound infection samples. *Staphylococcus aureus*60 (29.4%) was the predominant organism isolated from surgical sites followed by *Pseudomonas aeruginosa* 40 (19.6%), *Klebsiella pneumoniae* 34 (16.6%), *Escherichia coli* 29 (14.2%) in the present study. *Staphylococcus epidermidis* 16 (7.8%) *Proteus mirabilis* 11 (5.3%), *Citrobacter koseri* 8 (3.9%), *Enterococcus* species 4 (1.9%), *Acinetobacter* species 2 (0.9%) were the other organisms isolated from SSIs.

Many studies also found *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the commonest isolate from the post-operative wound infection which is concordance with the present study.[17-19]

Table.1 Distribution of culture positive cases according to spectrum of bacterial isolates

Sl.No.	Organisms	Total No (%)
1	*Staphylococcus aureus*	60 (29.4)
2	*Pseudomonas aeruginosa*	40 (19.6)
3	*Klebsiella pneumoniae*	34(16.6)
4	*Escherichia coli*	29(14.2)
5	*Staphylococcus epidermidis*	16(7.8)
6	*Proteus mirabilis*	11(5.3)
7	*Citrobacter koseri*	8(3.9)
8	*Enterococcus species*	4(1.9)
9	*Acinetobacter species*	2 (0.9)
	Total	204

Table.2 Antibiotic sensitivity pattern of Gram positive organisms

Sl. No.	Organisms Total-80	AMC (%)	G/HLG (%)	Ak (%)	Ctr (%)	Cf (%)	Co (%)	E (%)	CD (%)	Lz (%)	Cn (%)	Va (%)
1	***Staphylococcus aureus* (60)**	20 (33.3)	54 (89.7)	58 (96)	31 (52.3)	13 (22.2)	28 (46.6)	40 (66.6)	45 (75)	60 (100)	48 (80)	**60 (100)**
2	***Staphylococcus epidermidis* (16)**	4 (25)	8 (50)	10 (62.5)	13 (81.2)	7 (43.7)	6 (37.5)	9 (56.2)	12 (75)	16 (100)	16 (100)	**16 (100)**
3	***Enterococcus species* (4)**	**0 (0)**	**2 (50)**	**2 (50)**	**2 (50)**	**3 (60)**	**2 (50)**	**4 (100)**	**4 (100)**	**4 (100)**	**4 (100)**	**4 (100)**

Abbreviations:Amp/sul-Ampicillin/sulbactum, G-Gentamicin,HLG-high level Gentamicin, Ak-Amikacin, Ctr-ceftriaxone,Cf-Ciprofloxacin, Co-Cotrimoxazole, E-erythromycin, CD- clindamycin,Lz-linezolid,Cn-cefoxitin, Va-vancomycin.

Table.3 Antibiotic sensitivity pattern of Gram negative organisms

Sl. No.	Isolates Total no isolates (124)	Amp/ sul (%)	G (%)	Ak (%)	cf (%)	Co (%)	Cpz/sul (%)	Ca (%)	Ce (%)	Ctr (%)	Cpm (%)	Pc (%)	Pt (%)	I (%)
1	***Pseudomonas aeruginosa (40)***	11 (27.5)	22 (55)	30 (75)	19 (47.5)	0 (100)	34 (85%)	33 (82.5)	32 (80)	34 (85%)	15 (37.5)	6 (14.2)	34 (85%)	40 (100)
2	***Klebsiellapneumoniae (34)***	7 (20.5)	19 (55)	21 (60)	19 (54.3)	15 (45.6)	23 (67.6)	20 (58)	21 (61.7)	20 (58)	25 (73.5)	11 (32.5)	31 (91.2)	34 (100)
3	***Escherichia coli (29)***	12 (21.6)	16 (55.6)	19 (64.5)	11 (37.5)	15 (52)	53 (92.9)	20 (68.9)	21 (72.4)	22 (75.8)	25 (86.2)	14 (48)	27 (94.5)	29 (100)
4	***Proteus mirabilis (11)***	3 (27.2)	5 (45.4)	6 (33.3)	4 (54.5)	4 (54.5)	7 (63.6)	11 (100)	11 (100)	11 (100)	11 (100)	4 (54.5)	11 (100)	11 (100)
5	***Citrobacter koseri (8)***	**2 (25)**	**4 (50)**	**5 (62.5)**	**4 (50)**	**4 (50)**	**6 (75)**	**8 (100)**	**8 (100)**	**8 (100)**	**8 (100)**	**4 (50)**	**8 (100)**	8 (100)

Abbreviations:Amp/sul-Ampicillin/sulbactum, G-Gentamicin, Ak-Amikacin,cf-ciprofloxacin, Co-Cotrimoxazole, Cpz/sul- cefeperazone-sulbactum, Ca-Ceftazidime, Ce-Cefotaxime,Ctr-ceftriaxone,cpm-cefepime,PtPiperacillin/tazobactam, I-Imipenem,

Table.4 Antibiotic sensitivity pattern of MRSA

Sl. No.	Organisms	AMC (%)	G (%)	Ak (%)	Ctr (%)	Cf (%)	Co (%)	E (%)	CD	Lz (%)	Cn (%)	Va (%)
1	***MRSA*(12)**	**0 (0)**	**7 (58.3)**	**9 (75)**	**10 (83.3)**	**6 (50)**	**0 (0)**	**10 (83.3)**	**11 (91.6)**	**12 (100)**	**0 (0)**	**12 (100)**

Abbreviations:Amp/sul-Ampicillin/sulbactum, G-Gentamicin, Ak-Amikacin, Ctr-ceftriaxone,Cf-Ciprofloxacin, Co-Cotrimoxazole, E-erythromycin, CD- clindamycin,Lz-linezolid,Cn-cefoxitin, Va-vancomycin.

Staphylococcus aureus 60 (29.4%) was the most common Gram positive bacteria followed by *Staphylococcus epidermidis* 16 (7.8%) and *Enterococcus* spp 4 (1.9%) isolated in SSI. 12 (20%) strains were of MRSA. All the MRSA strains are 100% sensitive to Vancomycin and Linezolid.

Among Gram negative isolates, *Pseudomonas aeruginosa* 40 (19.6%) was the most common

organism isolated in SSI followed by *Klebsiella pneumoniae* 34 (16.6%), *Escherichia coli* 29 (14.2%). It has high sensitivity towards Imipenem(100%), Piperacillin-tazobactum (85%).

In the present study members of Enterobacteriaceae family showed high sensitivity to Imipenem, Piperacillin-tazobactum and amikacin.

In the present study observed 13.2% of the *Escherichia coli* and 20.5% of *Klebsiella pneumoniae* to be ESBL producers which is comparable with other studies.[20,21]

There is a gradual increase in the emergence of antibiotic resistant organisms in surgical patients. The majority of the Gram-negative isolates are sensitive to Imipenem and Piperacillin-tazobactum while Gram positive isolates being sensitive to vancomycin and Linezolidand partly to fluoroquinolones. These findings evident the need for effective infection control and rational use of antimicrobial agents leading to minimize infection rate and emergence of drug resistance. The surgical site infections are the commonest nosocomial infection in hospital acquired infection. The present study showed the commonest bacteria responsible for the surgical site infections like *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* Hence the study concludes that strict major needs to be taken to avoid such surgical site infections. Also the treatment of this may leads to the antibiotic resistance. The data in the present study may be useful in choosing the effective therapy against the isolates from surgical site infections.

Ethical approval

The study was approved by the Institutional Ethics Committee

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How to cite this article:

Anusuya Devi Devaraju and Latha Roy, S. 2021. Antimicrobial Susceptibility Pattern of Bacterial Isolates from Surgical Site Infections in A Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 10(11): 369-375. doi: https://doi.org/10.20546/ijcmas.2021.1011.042