Evaluation of Bacteriological Profile and Antimicrobial Susceptibility Pattern of Surgical Site Infections (SSIs) in Different Surgeries – An Observational Study from Ahmedabad, India

Nidhi Girishkumar Sathwara[1], Khushbu Rajendrakumar Modi[2], Kunal Girishkumar Sathwara[3], Chintan Chandrakantbhai Dalwadi[4], Manan Chandrakantbhai Dalwadi[5]

[1]Department of Microbiology, Smt. NHL MMC and Sardar Vallabhbhai Patel Institute of Medical Sciences & Research Hospital, Ahmedabad, Gujarat, India. [2]Department of Dermatology, LG Hospital and Medical College, AMC MET, Ahmedabad, Gujarat, India. [3]Department of Surgery, V.S. General Hospital, Ahmedabad, Gujarat, India. [4]Department of Microbiology, GMERS Medical College, Junagadh, Gujarat, India. [5]Department of Prosthodontics, Narsinhbhai Patel, Dental College & Hospital, Visnagar, Gujarat, India.

ABSTRACT

BACKGROUND

Emergence of multidrug-resistant bacterial pathogens in hospitals and associated risk factors are a strenuous task for clinicians to treat surgical site infections (SSIs). Isolation of multidrug-resistant organisms is an existing problem with a rising trend in Indian hospitals. We wanted to study the microbial profile, their susceptibility pattern, risk factors of SSIs, and revise the antibiotic prophylaxis policy to reduce injudicious use of antimicrobial agents.

METHODS

The present prospective observational study included 1073 post-operative patients of different surgeries held at a Tertiary Care Hospital in western India from July 2017 to August 2018. Samples were collected using a sterile cotton swab stick and processed as per standard operative procedures in appropriate culture media and susceptibility testing was done using the Kirby-Bauer disc diffusion technique. After incubation plates were examined under the reflected light they were interpreted according to clinical and laboratory standards institute (CLSI) guidelines.

RESULTS

Among 1073 samples, bacteriologically proven surgical site infection was identified in 63 (5.87 %) patients. In the present study, the predominant organism isolated was *E. coli* (28.57 %), followed by Klebsiella spp. (23.81 %), *Staphylococcus aureus* (19.05 %), *Pseudomonas aeruginosa* (17.46 %), Acinetobacter spp. (9.52 %), and *Proteus mirabilis* (1.59 %). Pan-antibiotic resistance was noted among 14 (27.45 %) gram-negative rods and 7 (58.33 %) methicillin-resistant *Staphylococcus aureus* strains were isolated.

CONCLUSIONS

Overall, resistance to the cephalosporin group of antibiotics and penicillin group has increased. So, rather than moving on to the higher generation antibiotics, aminoglycosides (amikacin/gentamycin) and fluoroquinolones (levofloxacin) are the better-preferred drugs.

KEYWORDS

Surgical Site Infections, Injudicious Use of Antimicrobial Agents, Antibiotic Prophylaxis Policy

Corresponding Author:
Dr. Chintan Chandrakantbhai Dalwadi,
Address: c/133, Someshwar Park-3,
Nr: Ambalal Park, Gate No - 4, Thaltej,
Ahmedabad - 380054, Gujarat, India.
E-mail: ccdalwadi78@gmail.com

DOI: 10.18410/jebmh/2021/379

How to Cite This Article:
Sathwara NG, Modi KR, Sathwara KG, et al. Evaluation of bacteriological profile and antimicrobial susceptibility pattern of ssis in different surgeries – an observational study from Ahmedabad, India. J Evid Based Med Healthc 2021;8(24):2017-2022. DOI: 10.18410/jebmh/2021/379

Submission 01-03-2021,
Peer Review 11-03-2021,
Acceptance 30-04-2021,
Published 14-06-2021.

BACKGROUND

Surgical site infection is defined as an infection occurring within 30 or 90 days after a surgery except for stitch abscess (or within 12 months if an implant is left in place after the procedure) and affecting either superficial or deep incisional infection or infections involving organ or body space.[1] Hospital-acquired surgical site infections are one of the serious health problems affecting hospitalized patients.[1] Globally SSI rates have been reported to range from 2.5 % to 41.9 %.[1] In recent years there has been a shift noted towards infection with antibiotic-resistant strains of both – gram-positive and gram-negative organisms, in SSI.[1]

While in today's era because of antimicrobial prophylaxis and infection control practices, SSIs remain a substantial cause of morbidity and mortality rate of 3 %. Of this, 75 % of the mortality rate has been directly related to SSI.[2] The battle between bacteria and their susceptibility to drugs is yet difficult tasks. The present task was carried out to monitor the rate of SSIs & revise the antibiotic prophylaxis policy by knowing microbial profile and their antimicrobial susceptibility pattern at the hospital & also to study the risk factors of SSIs using the National Nosocomial Infections Surveillance system (NNIS).

METHODS

The present prospective observational study was carried out in post-operative patients (1073) who had undergone different surgeries held at the Tertiary care teaching center, western side of India from July 2017 to August 2018. All post-operative patients who developed symptoms of SSIs from general surgery, obstetrics & gynaecology, and orthopaedic wards in the hospital were included in the study. Patients with known pre-operative & other than post-operative bacterial wound infections were excluded from the study.

The proforma includes age, sex, and type of-surgery (emergency or elective), anaesthesia, and wound. ASA score associated risk factors & duration. The entire study is divided into three parts:

1. Sample collection
2. Bacteriological processing and identification
3. Antibiogram

Sample Collection

Samples (like wound aspirates, exudates and discharge from the depth of wound) of post-operative infections were collected from patients with the help of two sterile swabs (one for gram stain and one for culture) by doctors or nursing staff with complaints of signs of inflammation, pain, swelling, & non-healing wound before antiseptic dressing then were properly labelled & delivered to the bacteriology section where these samples were processed.[3,4] All patients were followed up, monitored & explained about the sign of SSIs till discharge in the post-operative ward. Samples with detail-filled LRF were received in Laboratory, ID-was generated by using LIS.

Processing of Samples

Direct Microscopy

Smears were prepared on a clean glass slide by rotating one swab, gram staining was done by standard technique[4] and examined under the light microscope which differentiates organisms into gram-positive cocci & gram-negative bacilli

Processing of Sample for Culture

All the samples were inoculated on MacConkey and blood agar then incubated overnight aerobically at 37°C in an incubator and results were read after 24 to 48 hours. All positive cultures were, identified by their characteristic colony morphology, compared gram staining from the direct and culture smears, and by the pattern of a biochemical reaction.[3,4] The conventional biochemical tests such as catalase, coagulase (Tube & Slide), mannitol fermentation for gram-positive bacteria and oxidase test, triple sugar iron test, citrate utilization test, phenylalanine deaminase test, urease test, indole test, methyl red test, Voges Proskauer test, sugar fermentation test, nitrate reduction test, acetamide test, amino acid decarboxylase test, oxidation/fermentation test for gram-negative bacteria.

Antibiogram of Isolates[3,4]

Kirby Bauer disk diffusion test for antimicrobial susceptibility testing (AST) was performed after bacterial growth on the mentioned agar plates.

Suspension

At least 3 - 5 well-isolated colonies were selected using a sterile cotton swab and were transferred into the normal saline tube and mixed well. The turbidity of the suspension was adjusted according to 0.5 % McFarland turbidity standard. A lawn culture was done on MHA using suspension dipped sterile cotton swab then antibiotic disc was applied & inverted plates incubated at 35 - 37°C for overnight aerobically.

Reading of Plates and Interpretation of Results

After incubation, plates were examined by the naked eye, with the help of antibiotic zone scale including the diameter of the discs which was noted referring to CLSI guidelines. The results were interpreted and reported as follows: Susceptible(S), Intermediate(I), Resistant (R) then special tests were done for detecting MRSA, MBL, ESBL, and AmpC.[5]

Quality Control

The reliability of the study findings was guaranteed by implementing quality control (QC) measures throughout the whole laboratory works. All materials, equipment, and procedures were adequately controlled, and each procedure was aseptically performed. Culture media were tested for

sterility during the lot changes or validation. International control bacteria strains, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 43300, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212**,** *Pseudomonas aeruginosa* ATCC 27853 were used according to the CLSI.

Detection OF Methicillin Resistance

A lawn culture was done on MHA using the suspension of *S. aureus* isolate tested for methicillin resistance by using 30 µg cefoxitin disk.[6] The *S. aureus* strains ATCC 25923 and ATCC 43300 were taken as negative and positive controls respectively. After incubation read zone of inhibition was ≤ 21 mm which indicates MRSA.

Detection of ESBL

Screening Test

After lawn culture of isolates, disks of Ceftazidime (30µg) and Cefotaxime (30µg) were placed on inoculated MHA plate which incubated at 35 ± 2°C for 16 to 18 hours. Ceftazidime ≤ 22mm or Cefotaxime ≤ 27mm was taken as an indicator of ESBL production confirmed by phenotypic combination disk test. After lawn culture of isolates disks of Ceftazidime (30 µg), Ceftazidime plus clavulanic acid (30 µg / 10 µg) were placed on the MHA plate. After incubation, an increase of ≥ 5 mm in zone diameter with the antibiotic tested in combination with acid versus tested alone was taken as ESBL producers.[7]

Detection of Amplified Cephalosporinase (AMPC)

Screening Test

An isolate was screened for Ampc β- Lactamases by Kirby-Bauer's disk diffusion method demonstrating reduced susceptibility to cefoxitin (30 µg) as ≤ 18mm zone of inhibition.

Confirmatory Test for AMPC β-Lactamase

Modified Three-Dimensional Test (MTDT)

Lawn culture of *E. Coli* ATCC 25922 was done on MHA plates, and cefoxitin (30 µg) disc was placed on the surface of the medium. Linear slits (3 cm long) were cut using a sterile blade up to a point 3 mm away from the edge of the cefoxitin disc. Wells of 8 mm diameter were made on the slits at a distance of 0.5 cm inside from the outer end of the slit using a sterile Pasteur pipette. The wells were loaded with organism's inoculum until the well was full. After incubation zone of inhibition of cefoxitin was taken as Ampc producers.[8]

Detection of Carbapenemase

Modified Hodge Technique

Lawn culture of *E. coli* ATCC 25922 to the MHA plate put ertapenem (10μg) disk in the center of the plate then streak test isolates from the edge of the disk to edge of the plate. Positive strain shows clover leaf appearance at the end of the streaking line towards the disk.

Detection of Class B carbapenemase by CDST

For the detection of carbapenemases, we use the phenotypic method combine disc synergy test (CDST). Streak a lawn of the test strain to a Muller Hinton agar plate. This method involves the use of two discs one with a carbapenem antibiotic (Imipenem 10mcg) and the other with carbapenem with an inhibitor (Imipenem with EDTA) an increase in the zone diameter around the disc with inhibitor by ≥ 5 mm over the carbapenem disc indicate metallo-beta lactamase production.

Colistin mic testing[4] Colistin powder 2µg/ml is incorporated into MHA plate then 32 - 36 inoculums of test organisms can be applied simultaneously to the agar surfaces using an inoculums loop wire. After incubation, if growth occurs, it indicates the organism is resistant to Colistin and such strains were sent to NCDC New Delhi under National Program for confirmation.

The antibiotics like gentamicin (10 μg), amikacin (30μg) ciprofloxacin & levofloxacin ((5 μg) each), and tetracycline & doxycycline ((30 μg) each) ampicillin (10 μg), ampicillin-sulbactam (20μg), co-trimoxazole (25 μg), cefuroxime (30 μg) and chloramphenicol (30 μg) were used for both gram-positive and gram-negative bacteria. Penicillin G (10 units), erythromycin (15 μg), clindamycin (2 μg) cefoxitin (30 μg), vancomycin, teicoplanin& linezolid (30 μg) each)) were used for gram-positive while piperacillin-tazobactam (110 μg), ceftazidime (30 μg), cefepime (30 μg) and imipenem (10μg) were used for gram-negative organisms. While cefotaxime (30μg) & cefoperazone (75 μg) used in Enterobacteriaceae and piperacillin (100 μg), ticarcillin (75μg), mezlocillin (25μg), netilmicin (30 μg) & cefepime-tazobactam (40 μg) used in Pseudomonas.

RESULTS

Among 1073 patients, SSIs were found in 63 (5.87 %). Among 63 cases, *E. coli* 18 (28.57 %), Klebsiella spp. 15 (23.8 %), *S. aureus* 12 (19.05 %), *Pseudomonas aeruginosa* 11 (17.46 %), Acinetobacter spp. 6 (9.5 %), *Proteus mirabilis* 1 (1.59 %).

Distribution of SSI According to Surgery

Among 131 laparotomy: 19 infected (14.5 %), 21 colo-rectal: 2 infected (9.52 %), 34 small intestinal: 2 infected (5.88 %), 42 cholecystectomy: 2 infected (4.76 %), 43 mastectomy: 2 infected (4.65 %), 56 appendectomy: 2 infected (3.57 %), 96 hernia: 3 infected (3.13 %), 37 thyroidectomy, 5 hydrocele, 24 total knee replacement and 15 total hip replacement: 0 infected (0.0 % each), 77 Plate/k wire: 7 infected (9.09 %), 39 implant removal: 2 infected (5.13 %), 37 spondylitis: 1 infected (2.70 %), 355 LSCS: 19 infected (5.35 %), 61 abdominal hysterectomy: 2 infected (3.28 %).

	Various Factors	Case	Infected	%
Age	0 - 20	89	6	6.74
	21 - 40	585	29	4.96
	41 - 60	287	19	6.62
	≥ 61	112	9	8.04
Pre-operative hospital stay (days)	0 TO 5	722	19	2.63
	6 TO 10	345	43	12.46
	> 10	6	1	16.67
ASA score	1	224	0	0.0
	2	545	18	3.30
	≥ 3	304	45	14.80
Risk index	0	214	3	1.4
	1	316	15	4.75
	2	505	34	6.73
	3	38	11	28.95
Type of OT	Elective	984	49	4.98
	Emergency	89	14	15.73
Type of anaesthesia	Spinal	846	36	4.26
	General	227	27	11.89
Wound type	Clean	389	3	0.77
	Clean contaminated	485	25	5.15
	Contaminated	154	23	14.94
	Dirty	45	12	26.67
Duration	0 - 1 hour	484	14	2.89
	1 - 2 hour	543	42	7.73
	> 2 hours	46	7	15.22
Risk factor	Anaemia	66	12	18.18
	Diabetes mellitus	83	21	25.30

Table 1. Various Risk Factors

Antimicrobial Susceptibility Pattern

In present study, *S. aureus* (N = 12) was resistant to (Penicillin G, ampicillin 9(75 %) each), (cefuroxime, ampicillin-sulbactam 8 (66.67 %) each), (Co-trimoxazole, ciprofloxacin, gentamycin 5 (41.67 %) each), (Levofloxacin, clindamycin 6 (50.0 %) each), (Chloramphenicol, tetracycline, doxycycline 2 (16.67 %) each), amikacin 4 (33.33 %), erythromycin 10 (83.3 %) and cefoxitin 7 (58.33 %) (MRSA strain) while sensitive to vancomycin, linezolid & teicoplanin (12 (0.0 %) each)

Gram negative organisms (N = 40) were highly resistant to ampicillin* 18 (94.73 %), cephalosporin: 2nd, 3rd & 4th generation 37 (92.5 %), 36 (90 %) & 35 (87.5 %) respectively, ciprofloxacin 33 (82.5 %), levofloxacin 32 (80 %), co-trimoxazole 30 (75 %), ampicillin-sulbactam** 26 (76.47 %) & piperacillin-tazobactam 29 (72.5 %) and aminoglycosides 26 (65.0 %), tetracyclin*** 25 (64.1 %), minocycline 25 (62.5 %) were relatively less resistant while chloramphenicol 23 (57.5 %), imipenem 16 (40 %) were relatively effective.

P. aeruginosa (N = 11) demonstrated high level of resistance to ciprofloxacin, piperacillin, ticarcillin, mezlocilli, ceftazidime (10 (90.90 %) each), aztreonam, gentamycin, tobramycin, amikacin, netilmicin (9 (81.81 %) each) & less resistance to cefepime, cefepime-tazobactam, piperacillin-tazobactam, levofloxacin (7 (63.63 %) each) while imipenem 3 (27.27 %) was relatively effective.

(*), (**), (***) indicate intrinsic resistance to (Klebsiella & Acinetobacter spp.), (Acinetobacter spp.), (Proteus spp.) respectively.

ESBL & AmpC producer respectively: among 18 *E. coli* 3 (16.67 %) & 8 (44.4 %) and among 15 Klebsiella spp. 2(13.3 %) & 3 (20 %). Carbapenemase produces among 34 Enterobacteriaceae 13 (38.2 %), detected by modified Hodge test and metallo-beta lactamases by CDST representing of class A, B, and Class D beta-lactamases and metallo-beta lactamases produce among 17 non-fermenters 5(29.41 %), detected by CDST representing class B beta-lactamases.

Multidrug Resistance Organism

Gram-negative rods: Pan-antibiotic resistance noted in 14 (27.45 %) isolates among them 2 *E. coli*, 9 Klebsiella spp. (most resistance isolate) & 3 *Pseudomonas aeruginosa*.

DISCUSSION

Comparison of the Rate of SSIs between the Present and Other Studies

General Surgical Procedure

2008-Umesh et al.[3] 30.70 %, 2010 - Mahesh c b et al.[9] 20.90 %, 2013 - Barnali kakati et al.[7] 7.44 %, 2018 - present study 6.88 %.

Obstetrics & Gynaecology Procedure

2008 - Jesus Molina[10] 5.26 %, Maria Roumbelaki RN[11] 5.33 %, 2018 - present study 5.05 %.

Abdominal Hysterectomy

NINSS& PHLS report[12] 2.40 %, present study 3.28 %, Jesus[10] 6.00 %, G Taylor[13] & Cecilia[12] (7.60 % each) & in LSCS: Olsen MA[14]5.00 %, present study 5.35 %, Tran Thach[15] 8.90 %, Cecilia[12] 22.50 %.

Orthopaedic Surgeries

(2005-Lilani SP, Daver GB[16] 8.95 %), (2014 Fahad A. Al-Mulhim, 1 Abdallah S. Alomran[17] 2.25 %), (2017- Dr. Amaradeep G, Dr. ManjappaCN[18] 4.43 %) & present study 5.21 %.

Risk Factor & SSIs Rate

In our study patients were divided into four age groups. The rate of SSI was the highest (8.04 %) in ≥ 61 years. Among 83 patients with diabetes mellitus, 21 had SSI (25.30 %). Prolonged pre-operative hospital stays (> 10 days) was found to be associated with a higher rate of SSI (16.67 %) as this leads to colonization with antimicrobial-resistant microorganisms.

Comparative studies for rate of SSI according to pre-operative hospital stay: Patel Sachin[19] 0 - 5 Day (5.5 %), 6 - 10 days (12.8 %), >10 days (33.3 %), Mahesh C B[9] 0 -5 day (13.93 %), 6 - 10 days (28.57 %), > 10 days (33.3 %) and present study: 0 - 5 day (2.63 %), 6 - 10 Days (12.46 %), > 10 days (16.67 %). The rate of SSI was higher in emergency surgery (15.73 %) than elective surgery (4.98 %), due to inadequacy of time for appropriate preoperative aseptic preparation & antibiotic prophylaxis.

Comparative studies for rate of SSI according to type of surgery: Mahesh C B[9] elective - 7.61 % & emergency - 21.05 %, Satyanarayana V[20] elective - 7.6 % & emergency - 25.2 %, Barnali Kakati[7] elective - 4.86 % & emergency - 15.2 %, Hariom Sharan[21] elective - 10.53 % & emergency - 19.44 %, Agrawal Amit[22] elective - 5.7 % & emergency - 28.6 %, present study elective – 4.98 % & emergency - 15.73 %.

Surgical sites were classified using CDC's criteria. The rate of SSI is almost doubled with dirty/infected surgical sites than contaminated surgical sites. The endogenous (found between clean and clean-contaminated wound) or exogenous (found between clean contaminated & dirty

wound) contamination of wounds by the organisms had a profound influence on the rate of SSIs.[9]

Study	Clean	Clean Contaminated	Contaminated	Dirty	Total
David h. Culver (1987-1999)[23]	2 %	3 %	6 %	7 %	--
Lilanispjangale N. (2003) ijmm[16]	3.03 %	22 %	--	--	8.9 %
Barnalikakati (2013)[7]	3.7 %	4.45 %	11 %	16.8 %	7 %
Mahesh C B (2010)[9]	11.5 %	23 %	38 %	57 %	20 %
N Nreddy (2013-2014)[24]	4.3 %	8 %	34.78 %	52 %	6.8 %
Present study (2018)	0.77 %	5.15 %	14.9 %	26.6 %	5.87 %

Table 2. Comparative Studies for the Rate of SSI According to Wound Type

	ASA Score (Based on the Rate of SSIs)		
	1	2	≥ 3
Culver[23]	1.5 %	2.1 %	5.5 %
Patel Sachin[19]	0.0	4.2 %	29.8 %
Present study	0.0	3.3 %	14.8 %

Table 3. Comparatives Study

In the present study, the SSI rate was higher (11.89 %) in general anaesthesia due to artificial respiration causes tissue hypoxia. The risk indices help in surveillance and infection control programs.[19]

Author/RI	Anderson[27]	NNIS Report[12]	Present Study
0	0.47 %	2.70 %	1.40 %
1	0.71 %	4.10 %	4.74 %
2	2.15 %	7.50 %	6.73 %
3	NA	NA	28.94 %

Table 4. Comparatives Studies for the Rate of SSI According to Risk Index (RI)

Comparatives studies for rate of SSI according to gram-positive/gram-negative organisms: 2003 - Hayath Kownhar[25] gram-positive: 41.9 % & gram-negative: 58 %, 2004 - Moataz abdel-Fattah[26] gram-positive: 31.8 % & gram-negative: 66.2 %, 2018 - present study gram-positive: 19.04 % & gram-negative: 80.95 %. *E. coli* predominates because the majority of surgeries involved intra-abdominal sites where gram-negative organisms were predominantly found.

Year	Study	*Staphylococcus aureus*	*E. coli*	*Pseudomonas aeruginosa*	Klebsiella spp.	Proteus spp.	Acinetobacter spp.
1986 - 1989	NNIS Report[28]	17 %	10 %	8 %	3 %	4 %	-
1990 - 1996	NNIS Report[28]	20 %	8 %	8 %	3 %	3 %	-
2012	Hariom Sharan[22]	31.5 %	10.5 %	15.7 %	26.3 %	5.6 %	10.53 %
2012	Sahane V[29]	22 %	31.2 %	25 %	-	-	-
2013	Ramesh[30]	16 %	20.8 %	16 %	15 %	-	-
2013	Barnali[7]	13.7 %	41 %	7.8 %	9.8 %	2 %	2 %
2018	Present study	19.05 %	28.57 %	17.46 %	23.8 %	1.59 %	9.52 %

Table 5. Comparatives Studies for the Rate of SSI According to Various Pathogens

Antibiotic Susceptibility of Various Organisms

All gram-negative isolates were found to be sensitive to imipenem (60 %) while resistance to ampicillin, ampicillin-sulbactam, ciprofloxacin & all generation of cephalosporins were 94.7 %, 76.5 %, 82.5 % & > 92.5 % respectively. Extended spectrum beta-lactamase (ESBL) production was detected in the present study 16.67 % strains of *E. coli* & 13.3 % of *K. pneumoniae*. In a study by David Agatha,[10] ESBL producing *E. coli* was 47.8 % & *K. pneumoniae* was 50 %.[3]

CONCLUSIONS

To decrease the emergence of multidrug-resistant organisms, the following factors must be taken into account - host, microbial, environmental, and continuous surveillance systems. Treating existing co-morbidities whenever possible and pre-operative hair removal with razor & antibiotic prophylaxis should be done within 30 minutes before surgery. Strict antimicrobial prophylaxis guidelines in patients of SSIs should be followed. In the present study, aminoglycosides (amikacin/gentamycin) & fluoroquinolones (levofloxacin) are preferred to combat SSIs for both gram-positive and gram-negative isolates. So, rather than moving on to higher-level antibiotics wait for antibiotic sensitivity results.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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