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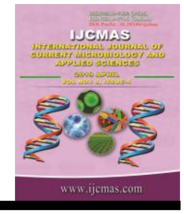

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Aerobic Bacteriological Profile and Antibiogram of Surgical Site Infections in a Tertiary Care Hospital in Kashmir

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A B S T R A C T

To determine the most common organisms causing surgical site infections and their antibiotic sensitivity pattern. This study was carried out retrospectively in the Department of Microbiology SKIMS Medical College and associated hospital, Srinagar for a period of two years (January 2017–December 2018). A total number of 194 pus samples of in-patients from different wards as well as of out-patients were processed using standard microbiological techniques. Of the 194 pus samples processed, 94 samples were culture positive (48.4%). *Escherichia coli* was the most commonly isolated organism (36%) followed by *Staphylococcus* spp. (34%), *Klebsiella* spp. (11.7%), *Acinetobacter* spp. (5.3%), *Enterococcus* spp. (4.25%), Coagulase negative Staphylococci (CONS) (3.1%), *Citrobacter* spp. (2.12%), *Pseudomonas* spp. (2.1%) and *Proteus* spp. (1.06%). All isolates of *Escherichia coli* were sensitive to Polymyxin-B and 90% sensitive to Imipenem. 14 *E.coli* isolates (41.17%) were multi-drug resistant. 100% of *Staphylococcus* and *Enterococcus* spp. were sensitive to Linezolid, Vancomycin and Teicoplanin. 15(46.87%) isolates of *Staphylococcus aureus* were Methicillin-resistant Staphylococcus aureus. Surgical site infections are an important cause of morbidity, mortality and economic burden among health care associated infections (HCAI). Absence of data, under-reporting and little or no surveillance of these infections are major areas of concern.

Introduction

Surgical site infections (SSI's) or infections at the site of surgical incision wounds are defined as microbial infiltration of the wounds occurring upto 30 days after surgery, or upto one year after surgery in patients receiving implants, and affecting either the incision or deep tissue above fascial layer at operation site.[1] These are the third most common cause of hospital-associated-infections worldwide and remain as one of the most important post-operative complications accounting for 10-20% of hospital cost.[2] Surgical procedures with complications have magnanimous impacts on health expenditures and increased duration of hospital stay.[3] Sepsis in surgery thus consistently remains a significant concern to the health professionals worldwide.[4]

Indiscriminate and injudicious use of

antibiotics has led to the progressive development of multidrug resistance that is an important cause of increased morbidity and mortality [5]

The multidrug resistant virulent organisms thriving in the hospitals are capable of causing disease in patients already compromised with injury, surgery, pregnancy, immune status and other co-morbid conditions over and above the use of broad spectrum antibiotics which reduce the normal microbial flora of the body [6,7]

The pathogens most frequently isolated from the pus samples of surgical sites are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella* spp.[8] All these bacteria are relevant in hospital environments that encounter intense selection pressure of extensive antibiotic therapy for a range of bacterial infections.

It is estimated that about half of the SSI`s are preventable using evidence based strategies. The prevention of SSI`s is increasingly important as the number of surgical procedures continue to rise. CDC has pointed out that the single most important measure for preventing the spread of nosocomial bacterial pathogens is effective hand washing. Handwash is recommended before and after contact with patients, before invasive procedures and after contact with contaminated inanimate objects [9]

In the present study, we aim to analyze cases of surgical site infections over a period of two years for the spectrum of pathogenic bacteria isolated and their antibiotic sensitivity pattern. This will be a step towards the development of institutional antimicrobial policies for empirical and culture guided therapies and also to formulate effective measures for control of hospital associated infections.

Materials and Methods

Study period and site

The present study was conducted in the Department of Microbiology Sher-i-Kashmir institute of Medical sciences Medical college and hospital, Bemina, Srinagar for a period of two years from Jan 2017 to Dec 2018.

Study type

Retrospective observational study

Sample size

194 samples collected from both out-patients as well as in-patients were processed.

Inclusion criteria

Samples from elective and emergency surgeries with

Signs of inflammation like tenderness, redness, sanguineous or purulent discharge or fever≥ 38^0C.[10]
Gaping wound.[10]

Exclusion criteria

Skin grafts.
Patient lost on follow-up.
Incision and drainage.

Methodology

Samples taken on swab or frank pus in syringes were processed using standard microbiological techniques. Following sequence of steps were taken in sample processing:

All samples were subjected to direct gram staining technique for presumptive evidence. This was followed by inoculation on routine laboratory media like blood agar, MacConkey agar and chocolate agar.[11]

After aerobically incubating the media at 37^0C for 24 hrs, any growth was studied for their colony characteristics including color, shape, size, texture or pattern of haemolysis if present.

Biochemical tests and spot tests were performed to confirm the organisms.[12]

Antimicrobial susceptibility testing of all isolates was performed using Kirby-Bauer's disc diffusion method following the CLSI guidelines.[13]

No growth obtained even after 48 hrs of incubation at 37^0C was labeled as sterile pus and were included in the present study.

Results and Discussion

A total of 194 pus samples were included in the study. Among these 194 samples, 94 samples showed significant growth and the rest of samples with no growth were labeled as sterile. Thus the total number of culture positive cases was 48.45%.

However, the percentage of culture positive cases were significantly more in emergency surgeries (54.8%) as compared to elective surgeries (39.50%). The results are depicted in Table 1.

The highest number of culture positive cases were observed from the department of orthopedics which mostly included trauma and road traffic accidents, followed by emergency surgeries like gut perforation, exploratory laparotomy following trauma, staging laparotomy etc.

A percentage wise distribution of culture positivity rates in various surgeries performed in our institute is given below in table 2.

Not much difference was observed in the percentage of surgical site infections among the two genders. Among the 194 cases studied, 103 were males (53.09%) and 91 were females (46.90%). However most of the males had undergone emergency surgeries due to injuries in road traffic accidents while most of the females had undergone elective surgeries mostly for gaenecological and obstetric reasons followed by cholecystectomy (Figure 1).

Table 3 highlights the most common organisms isolated in our study. *E coli* (36%) was observed to be the most frequent isolate followed by *Staphylococcus aureus* (34%), *Klebsiella* spp. (11.7%), *Acinetobacter* spp. (5.3%), *Enterococcus* spp. (4.25%), CONS (3.1%), *Pseudomonas* spp. (2.1%), *Citrobacter* spp. (2.12%) and *Proteus* spp. (1.06%). Most of these bacteria isolated are known causes of hospital acquired infections.

A total of 39 gram-positive organisms (41.48%) were isolated and their antibiotic sensitivity pattern determined. All isolates of *Staphylococcus aureus*, *Enterococcus spp*. and Coagulase negative Staphylococci were found sensitive to Teicoplanin, Vancomycin and Linzolid. However a considerable number of isolates of *Staphylococcus aureus* were found resistant to β lactams, aminoglycosides, and fluoroquinolones. The antibiotic sensitivity pattern of Gram positive bacterial isolates is given below in Table 4.

Most of the isolates of *Escherichia coli* and *Klebsiella* spp., the two most frequently isolated Gram negative species in our study, were resistant to Amoxy-clav, Ceftriaxone, Cefepime, Ciprofloxacin, Ofloxacin and Cotrimoxazole. On the other hand, sensitivity to Piperacillin-Tazobactum, Amikacin, Tobramycin, Imipinem and Polymyxin B was considerably higher. The antimicrobial sensitivity of Gram negative isolates is given below in Table 5.

Table.1 Culture positivity rates from surgical site pus samples

Type of surgery	No of pus samples processed	Culture positive cases	Percentage
Emergency	113	62	54.8%
Elective	81	32	39.50%
Total	194	94	48.45%

Table.2 Percentage of culture positive cases in various surgeries

Type of surgery	Cases processed	Culture positive	Percentage
Implants (Orthopaedic)	29	15	51.72%
Non-implants (Orthopaedic)	28	19	67.85%
Appendectomy	28	17	60.71%
Cholecystectomy	31	10	32.25%
Hernioplasty	13	4	30.76%
Others including gut perforation/laparotomy/ breast surgeries/staging laparotomy.	24	16	66.66%
LSCS	27	9	33.33%
Non-LSCS	14	4	28.57%

Table.3 Aerobic bacterial culture isolates with culture positive rates

Organisms isolated	No. of isolates	Percentage
Escherichia coli	**34**	**36%**
Staphylococcus aureus	**32**	**34%**
***Klebsiella* spp.**	**11**	**11.7%**
***Acinetobacter* spp.**	**5**	**5.3%**
***Enterococcus* spp.**	**4**	**4.25%**
Coagulase negative Staphylococcus	**3**	**3.1%**
***Pseudomonas* spp.**	**2**	**2.1%**
***Citrobacter* spp.**	**2**	**2.12%**
***Proteus* spp.**	**1**	**1.06%**

Table.4 Antibiotic sensitivity profile of gram-positive organisms isolated

S.no.	Antimicrobials	*Staphylococcus aureus* (n=32)	*Enterococcus* spp. (n=4)	CONS (n=3)
1	Penicillin	11 (34.37%)	0	0
2	Ampicillin	nt	1 (25%)	0
3	Cefoxitin	15 (46.87%)	nt	2 (66.66%)
4	Cefazolin	18 (56.25%)	nt	2 (66.66%)
5	Amox-clav	19 (59.37%)	0	0
6	Cotrimoxazole	24 (75%)	3 (75%)	3 (100%)
7	Amikacin	25 (78.12%)	1 (25%)	2 (66.66%)
8	Gentamycin	22 (68.75%)	2 (25%)	2 (66.66%)
9	Erythromycin	21 (65.62%)	nt	1 (33.33%)
10	Azithromycin	22 (68.75%)	nt	2 (66.66%)
11	Teicoplanin	100 (100%)	4 (100%)	4 (100%)
12	Ciprofloxacin	17 (53.12%)	2 (50%)	1 (33.33%)
13	Ofloxacin	19 (59.3%)	nt	2 (66.66%)
14	Levofloxacin	20 (62.5%)	3 (75%)	2 (66.66%)
15	Clindamycin	26 (81.25%)	nt	2 (66.66%)
16	Vancomycin	100 (100%)	4 (100%)	3 (100%)
17	Linezolid	100 (100%)	4 (100%)	3 (100%)

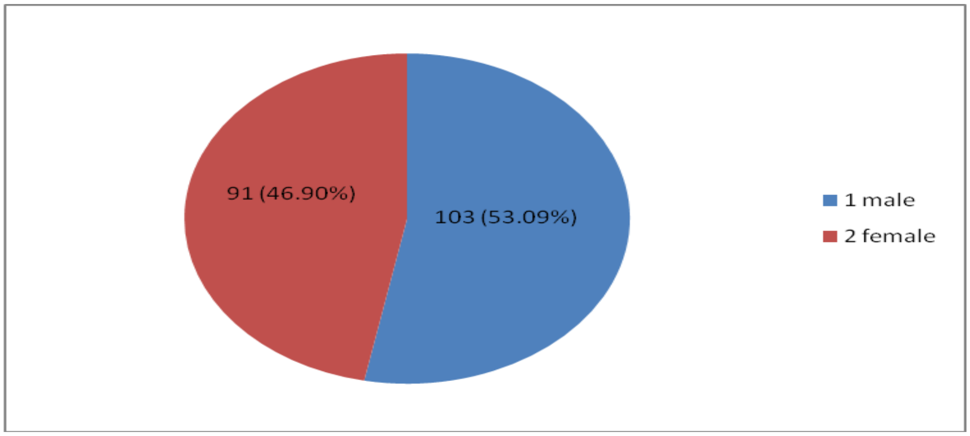
nt=not tested, CONS=Coagulase negative Staphylococcus

Table.5 Antibiotic sensitivity profile of gram-negative organism

S.no.	Antimicrobial	*E coli* n= 34	*Klebsiella* spp. n=11	*Acinetobacter* spp. n=5	*Pseudomonas* spp. n=2	*Citrobacter* spp. n=2	*Proteus* spp. n=1
1	Ampicillin		0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
2	Amox-clav	6 (17.6%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
3	Ceftriaxone	9 (26.47%)	3 (27.27%)	0 (0.00%)	nt	0 (0.00%)	1 (100%)
4	Cefepime	11 (32.35%)	2 (18.18%)	0 (0.00%)	0 (0.00%)	1 (50%)	
5	Ceftazidime	16 (47.05%)	2 (18.18%)	0 (0.00%)	1 (50.00%)	1 (50%)	1 (100 %)
6	Ceftazidime/ clauvanic acid	19 (55.88%)	4 (36.36%)	0 (0.00%)	2 (100.00%)	1 (50%)	1 (100%)
7	Cefprome			0 (0.00%)	0 (0.00%)	1 (50%)	0 (0.00%)
8	Pipercillin/ tazobactam	24 (70.58%)	7 (63.6%)	0 (0.00%)	2 (100%)	1 (50%)	1(100%)
9	Gentamycin	22 (64.7%)	4 (36.36%)	2 (40%)	1 (50%)	1 (50%)	
10	Amikacin	30 (88.2%)	7 (63.6%)	4 (80%)	1 (50%)	1 (50%)	1 (100%)
11	Tobramycin	20 (58.8%)	6 (54.54%)	2 (40%)	1 (50%)	1 (50%)	1 (100 %)
12	Ciprofloxicin	4 (11.76%)	3 (27.27%)	1 (20%)	1 (50%)	1 (50%)	1 (100%)
13	Ofloxacin	10 (29.41%)	4 (36.36%)	2 (40%)	0 (0.00%)	1 (50%)	0 (0.00%)
14	Meropenem	23 (67.64%)	3 (36.36%)	3 (60%)	1 (50%)	0 (0.00%)	0 (0.00%)
15	Imepenem	31 (91.1%)	11 (100%)	3 (60%)	1 (50%)	1 (50%)	1 (100 %)
016	Cotrimoxizole	9 (26.47%)	6 (54.54%)	0 (0.00%)	nt	0 (0.00%)	0 (0.00%)
17	Polymixin B	34 (100%)	11 (100%)	5 (100%)	2 (100%)	2 (100%)	0 (0.00%)
18	Tigecycline	nt	nt	5 (100%)	nt	nt	0 (0.00%)

nt=not tested, n=total number of isolates tested

Fig.1 Gender distribution of culture positive cases



The infection rate is higher in patients who underwent emergency surgeries than those who underwent elective surgeries. This is in concordance with studies by Lilani SP et al and Anvikar AR et al.[10,14]

The highest number of culture positive cases were from orthopedic surgeries which mostly included trauma and RTA's, followed by emergency surgeries like gut perforation, exploration laparotomy following trauma, staging laparotomy etc.

Of the 194 samples processed, 94(48.45%) showed bacterial growth after 24 hours of aerobic inoculation. The low percentage of culture positivity may be because of the reason that patients were already on empirical therapy that rendered pus samples sterile and secondly anaerobes were not tested because of unavailability of anaerobic culture. The percentage of culture positive cases were significantly more in emergency surgeries (54.8%) as compared to elective surgeries (39.5%) which is similar to some other studies.[15,16,17]

E coli (36%) was the most frequently isolated pathogen in this study. This was followed by *Staphylococcus aureus* (34%), *Klebseilla* spp. (11.7%), *Acinetobacter* spp. (5.3%), *Enterococcus* spp. (4.25%), CONS (3.1 %), *Pseudomonas* spp. (2.1 %), *Citrobacter* spp. (2.12%) and *Proteus* spp. (1.06%). Similar spectrum of bacterial profile was observed in some other studies.[16,18,19]

Gram negative bacilli showed 100% sensitivity to Polymixin-B, which is in concordance with most of the studies. *E coli* showed maximum sensitivity to Imipenem (91.1%). Two multidrug resistant strains of *E coli* were sensitive only to Polymixin-B. *Klebseilla* spp. was 100% sensitive to Imipenem and 100% resistance to amoxicillin-clavulanic acid. *Acinetobacter* spp. were 100% sensitive to Tigecycline followed by 80% sensitivity to Tobramycin. These drugs should however be used in selected cases and must be kept as reserve drugs for risk of acquired resistance against them.

Pseudomonas spp. were 100% sensitive to Pipercillin-Tazobactam and ceftazidime-clavulanic acid but 50% of isolates were found resistant to Carbapenems and Aminoglycosides. that was in concordance to some studies.[20,21]

All the gram-positive organisms were sensitive to Vanomycin (100%), Linezolid (100%) and Teicoplanin (100%) which is in concordance to the study by V.Singh et al**.**[19]

A high susceptibility rate of *Staphylococcus aureus* was also observed to Clindamycin (81.25%), Amikacin (78.12%), Cotrimoxazole (75%), Gentamycin (68.75%) and Azithromycin (68.75%).

Of the 32 isolates of *Staphylococcus aureus* 15 (46.87%) were identified as methicillin resistant. This high rate of isolation of MRSA was in concordance with few other studies.[22,23]

All 3 isolates of CONS were isolated from orthopedic implants. However, the most frequent isolation of *Escherichia coli* (a normal gut flora) and *Staphylococcus aureus* (a normal skin commensal) from surgical site wound samples necessitates the need for strict aseptic surgical procedures and hospital infection control policies.

In conclusion, the rapid rise in antibiotic resistance in microorganisms due to selection pressure and production of extended spectrum β lactamases, necessitates a proper antibiotic stewardship programme and development of Institutional antibiotic policies in hospitals. Restricted antimicrobial formalaries should be used only in selective situations under the strict guidance of Hospital Infection Control Committee.

Training of nursing staff, technicians in postoperative wards regarding maintenance of strict aseptic environment and interaction between clinicians and microbiologists needs to be emphasised. Protocols should be developed for preoperative workup and postoperative care to control risk factors causing surgical site infections.

Conflict of interest

The authors do not have any conflict of interest.

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