BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF AEROBIC WOUND INFECTION ISOLATES IN TERTIARY HEALTH CARE CENTRE

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Abstract

Background: Health care associated infection possess major problem for both doctor as well as patients. Health care associated infection prolongs hospital stay which leads to financial burden to the patients. Among health care associated infections, surgical site infections are the second most common after Urinary tract infection. **Material and Methods:** The present study included a total of 102 cases of wound infection who attended surgery out patients in NIMS Hospital. All patients with clinical evidence of sepsis were included during the study period. The samples were collected from the depth of the wound with strict asepsis. Two sterile cotton swabs from each sample were obtained before the wound was cleaned with antiseptic solution. Samples were taken from the patients during the period of surgical wound dressing without contamination with skin commensals and transported to the laboratory immediately. Methods: A total of 204 wound swabs were collected from patients with wound infections and were processed according to the standard microbiological procedures. **Results:** In our study, we found 82 patients were infected by the single organism whereas, 20 patients were infected by multiple organisms. Among three most commonly bacterial isolates *E. coli* (29.5 %), *Pseudomonas* (25.5 %), *S. aureus* (19.7 %) gram negative organisms had a higher prevalence. **Conclusion:** Increasing resistance to antimicrobials increases the risk of morbidity and mortality; therefore there is urgent need of implementation of measures to restrict the health care associated infection. Rational use of antimicrobials, proper hygiene, and strict asepsis should be applied in all health care.

Keywords: Health care associated infection, wound swabs, ESBL, MRSA, Staphylococcus aureus, skin and soft tissue infections (SSTI`S).

INTRODUCTION:

Infections which occur during the time of hospital stay and were not present or in incubating stage, during the time of hospital admission are considered as health care associated infections. **(1)** Health care associated infection possess major problem for both doctor as well as patients. Health care associated infection prolongs hospital stay which leads to financial burden to the patients. It has been reported that in United States of America the death frequency is about 88,000 every year despite of estimated cost of management of health care associated infections is about 4.5 billion dollar.**(2)** Among health care associated infections, surgical site infections are the second most common after Urinary tract infection.**(3)**

Surgical site infections have plagued surgeons since time immemorial. **(4)** Most of these surgical site infections are superficial. The pathogenesis of colonization, invasion and infection of pathogenic micro-organisms is due to the disruption of the superficial barrier which is either skin or mucous membrane at the site of infection as well as a large amount of necrotic tissue and exudates which is present on the surface of infection site, provides a good medium for the colonization of micro-organisms. Wound infection can be caused by different type of organisms ranging from gram positive organisms like *Staphylococcus aureus, CoNS, Enterococcus spp. to Gram negative organisms like Pseudomonas aeruginosa, Escherichia coli, Klebsiella spp, proteus spp, acinetobacter spp, enterobacter spp and Serratia marcescens* depending upon the prevalence of organism in the specific community. Each hospital has its own bacterial flora to which patients are at risk for acquiring health care associated infection. Among these organisms *Staphylococcus aureus* was the most common causative pathogen for wound infection but it has been replaced by Gram negative organisms.**(3)**

Antibiotics reduce the frequency of hospital acquired infections but due to multidrug resistance, wound infections and other post-operative infections possess a major problem for the treating doctors. Multi-antibiotic resistance has been noted in Gram positive organisms like methicillin-resistant *S. aureus* and in Gram negative bacilli *P. aeruginosa and Acinetobacter* spp. Determination of the causative organism is very important in the final choice of antibiotics. A working knowledge of the most likely causative organism and their antibiotic sensitivity provides a great help in combat with wound infections and antibiotic resistance. The department of microbiology serves very important function in diagnosing and treating health care associated infections, which helps the treating doctor in choosing the accurate antibiotics. Due to all these implications, this study was conducted to determine the aerobic bacterial wound infection isolates in a tertiary health care centre and describing their antibiogram, which would enable the determination of empirical antibiotic strategies for the early treatment of the infection and prevention of drug resistance.

MATERIAL AND METHODS

Present study is a hospital based prospective study. Study was conducted over a period of 6 months from sep. 2015 to Feb. 2016 at collaboration of surgery and microbiology department of our institute. The present study included a total of 102 cases of wound infection who attended surgery out patients in NIMS Hospital. **Inclusion criteria**: Patients of all age groups except neonates, presence of wound infection, patients giving informed consent to participate. **Exclusion criteria:** Neonates, infection of episiotomy, Burn injury and donor sites of SSG, refusal to give consent for participating in the study. **Sampling technique**: all patients with clinical evidence of sepsis were included during the study period. The samples were collected from the depth of the wound with strict asepsis. Two sterile cotton swabs from each sample were obtained before the wound was cleaned with antiseptic solution. Samples were taken from the patients during the period of surgical wound dressing without contamination with skin commensals and transported to the laboratory immediately. **Methods**: A total of 204 wound swabs were collected from patients with wound infections and were processed according to the standard microbiological procedures. **Direct Microscopy:** After receiving swabs in laboratory swab 1st was used for direct microscopy. The smear was prepared by rolling the swab stick on a clean glass slide, which was air dried, heat fixed and Gram's staining was done. Smear was screened for pus cells, Gram reaction, morphology, number and arrangement of the organisms was noted. **Culture for Aerobic Organisms**: The 2nd swab was used for the culture. Swab was inoculated onto 5% sheep blood agar and MacConkey agar plate by rolling the swab and streaking method. After 24 hours of incubation smear was prepared from isolated colonies and Gram's were done for identification, segregation into Gram positive and Gram negative. The organisms were further identified up to species level by biochemical and physiological tests. The antibiotic susceptibility was done by using the Kirby-Bauer disc diffusion method. Detection of ESBL was done by Disc diffusion method by using Ceftazidime 30µg and Ceftazidime-clavulanic acid 30µg/10µg. MBL detection was done by Zone enhancement with Ethlyene Diamine Tetra Acetic acid (EDTA) 5µl impregnated imipenem 10µg and ceftazidime 30µg disks.

RESULTS

This study was conducted over a period of 6 month in NIMS Hospital. In present study we found total number of isolates were 102. Among 102 isolates maximum numbers of isolates were E. coli (29.5%) followed by *Pseudomonas spp.* (25%), *S.aureus* (19.7%).

Table 1: ORGANISMS AND THEIR FREQUENCY IN WOUND INFECTION

ORGANISMS	F REQUENCY	PERCENT (%)
S. aureus	20	19.7
CoNS	6	5.9
Enterococcus	2	1.9
E. coli	30	29.5
Klebsiella	12	11.9
Pseudomonas	26	25.5
Acinetobacter spp	2	1.9
Proteus spp.	2	1.9
Enterobacter spp.	1	0.9
Citrobacter spp.	1	0.9

CoNS- Coagulase negative Staphylococci

Table 2: DISTRIBUTION OF THE ORGANISMS IN PURE GROWTH AND MIXTURES IN WOUND INFECTION

ORGANISMS (n=102)	PURE (n=82)	MIXTURE (n=20)
S. aureus (n=20)	17	3
CoNS (n=6)	5	1
Enterococcus (n=2)	2	Nil
E. coli (n=30)	24	6
Klebsiella (n=12)	10	2
Pseudomonas (n=26)	22	4
Acinetobacter spp (n=2)	1	1
Proteus spp. (n=2)	1	1
Enterobacter spp. (n=1)	Nil	1
Citrobacter spp. (n=1)	Nil	1

Among pure and mixed culture maximum numbers of isolates were E. coli (n = 24) followed by *Pseudomonas* (n=22), *S.aureus* (n=17)

Table 3: SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCI

	***S. aureus* (n=20)**		**CoNS (n=6)**		***Enterococcus* (n=2)**	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Penicillin	2 (10%)	18(90%)	2(33.3%)	4(66.7%)	0(0%)	2(100%)
Cloxacillin	18(90%)	2(10%)	4(66.7%)	2(33.3%)	2(100%)	0(0%)
Erythromycin	6(30%)	14(70%)	4(66.7%)	2(33.3%)	1(50%)	1(50%)
Cephalexin	4(20%)	16(80%)	2(33.3%)	4(66.7%)	1(50%)	1(50%)
Amikacin	12(60%)	8(40%)	5(83.3%)	1(16.7%)	2(100%)	0(0%)
Gentamicin	8(40%)	12(60%)	1(16.7%)	5(83.3%)	1(50%)	1(50%)
Ciprofloxacin	2(10%)	18(90%)	1(16.7%)	5(83.3%)	2(100%)	0(0%)
Cotrimoxazole	8(40%)	12(60%)	0(0%)	6(100%)	1(50%)	1(50%)
Vancomycin	20(100%)	0(0%)	6(100%)	0(0%)	2(100%)	0(0%)

Table 4: SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI

	***E. coli* (n=30)**		***Klebsiella* spp. (n=12)**		***Proteus* spp. (n=2)**	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	2(6.7%)	28(93.3%)	0(0%)	12(100%)	0(0%)	2(100%)
Cefotaxime	7(23.3%)	23(76.7%)	5(41.7%)	7(58.3%)	0(0%)	2(100%)

Ceftazidime	8(26.7%)	22(73.3%)	5(41.7%)	7(58.3%)	1(50%)	1(50%)
Amikacin	18(60%)	12(40%)	6(50%)	6(50%)	1(50%)	1(50%)
Gentamicine	7(23.3%)	23(76.7%)	3(25%)	9(75%)	0(0%)	2(100%)
Ciprofloxacin	7(23.3%)	23(76.7%)	4(33.3%)	8(66.7%)	0(0%)	2(100%)
Cotrimoxazole	6(20%)	24(80%)	3(25%)	9(75%)	0(0%)	2(100%)
Chloramphenicol	4(13.3)	26(86.7%)	5(41.7%)	7(58.3%)	1(50%)	1(50%)

Table 5: SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI

	***Acinetobacter* spp. (n=2)**		***Citrobacter* spp. (n=1)**		***Enterobacter* spp. (n=1)**	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin	0(0%)	2(100%)	0(0%)	1(100%)	1(100%)	0(0%)
Cefotaxime	1(50%)	1(50%)	0(0%)	1(100%)	0(0%)	1(100%)
Ceftazidime	1(50%)	1(50%)	1(100%)	0(0%)	1(100%)	0(0%)
Amikacin	2(100%)	0(0%)	0(0%)	1(100%)	1(100%)	0(0%)
Gentamicine	1(50%)	1(50%)	0(0%)	1(100%)	0(0%)	1(100%)
Ciprofloxacin	2(100%)	0(0%)	0(0%)	1(100%)	0(0%)	1(100%)
Cotrimoxazole	1(50%)	1(50%)	0(0%)	1(100%)	0(0%)	1(100%)
Chloramphenicol	1(50%)	1(50%)	1(100%)	0(0%)	0(0%)	1(100%)

Table 6: SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE BACILLI

	***Pseudomonas* spp. (n=26)**	
Antibiotic	Sensitive	Resistant
Cefotaxime	4(15.4%)	22(84.6%)
Ceftazidime	13(50%)	13(50%)
Amikacin	14(53.8%)	12(46.2%)
Gentamicin	9(34.6%)	17(65.4%)
Ciprofloxacin	3(11.5%)	23(88.5%)
Polymyxin B	20(76.9%)	6(23.1%)
Pepracillin	8(30.8)	18(69.2%)
Carbenicillin	14(53.8)	12(46.2%)

DISCUSSION

In the field of surgery wound infections poses a major problem. Advances in management of wound infection have not completely controlled this problem. **(5)** Factor which favors the wound infections are remote site infection, diabetes mellitus, use of steroids, colonization of microorganisms, smoking, old age and obesity, poor nutrition and prolonged hospitalization, drug resistance. Active surveillance on health care associated infection and formulating antibiotic policy can reduce the risk of health care associated infections.

In our study samples were taken from the department of surgery, we found 82 patients were infected by the single organism whereas, 20 patients were infected by multiple organisms. Among three most commonly bacterial isolates *E. coli* (29.5 %), *Pseudomonas* (25.5 %), *S. aureus* (19.7 %) gram negative organisms had a higher prevalence. In Agarwal et al **(6)** and Anvikar et al studies, **(3)** most prevalent organisms were gram negative bacilli which support our study. Among *E. coli* 30 (29.5%), 24 (80%) isolates grew in pure culture whereas 6 (20%) were grew in mixed growth. Out of 30 *E. coli* 12(40%) were ESBL producers. Amikacin, Ceftazidime and cefotaxime were the most effective antimicrobial agents against this organism. Among *Pseudomonas* spp. 26 (25.5%), 22 (84.6%) grew in pure culture whereas 4 (15.4%) grew in mixed growth. Among 26 *Pseudomonas* 10 (38.4%) were MBL producers. Out of 20 S. aureus 17 were grew in mixed culture whereas 3 were grew in pure culture. Out of 20, 14 were MRSA and out of 14, 13 were grown in pure culture. Vancomycin shown highest number of sensitivity to *S. aureus* (100%). Other drugs which were sensitive to *S. aureus* were cloxacillin (90%), Amikacin (60%), Clotrimazole (40%). In Kowli et al **(7)** also showed the similar results which supports our study. Among resistant drugs penicillin (90%), Ciprofloxacin (90%) showed maximum resistance followed by cephalexin (80%) and Erythromycin (70%). Higher percentage of resistance may be due to irrational use of antibiotics.

CONCLUSION

After urinary tract infection, wound infection is most common health care associated infection. This study determined the bacteriological profile of wound infection. The most common isolates in our study were *E. coli*, *Pseudomonas* and *S. aureus*. Most common organism isolated from pure culture was *S. aureus* followed by *Pseudomonas* and *E. coli*. Vancomycin was uniformly sensitive to all S. aureus. After vancomycin, cloxacillin was the second best drug followed by Amikacin and Clotrimazole. Among gram positive organisms maximum resistance were see against penicillin, ciprofloxacin and cephalexin. Among *Pseudomonas* most uniformly sensitive drugs were Polymyxin B followed by Amikacin and Ceftazidime, whereas ciprofloxacin was most common resistant drug for *Pseudomonas* followed by cefotaxime and pepracillin. Increasing resistance to antimicrobials increases the risk of morbidity and mortality; therefore there is urgent need of implementation of measures to restrict the health care associated infection. Rational use of antimicrobials, proper hygiene, and strict asepsis should be applied in all health care.

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