Antimicrobial susceptibility pattern of bacterial isolates of hospital acquired infection in burn unit at MBS hospital,Kota. Rajasthan.

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Abstract

Aims- To established the hospital acquired infection in aerobic bacterial isolates from burn wound infection and treatment with antibiotic susceptibility pattern. Methods- A retrospective study was conducted the burn unit at MBS Hospital and Kota medical college Kota, Rajasthan. One hundred burn patients have investigated a bacterial profile of burn wound infections. The specimen was collected from the surface area of burn ward and wound swab of burn patients. The organisms were isolated and identified with the biochemical test. Further using antibiotic susceptibility pattern for a test of the bacterial isolates. Results- Lack of uniform antibiotic policy and indiscriminate use of antibiotics may have lead to emergence of resistant bacterial strain. In our study Amikacin, meropenom, gentamycin, imipenem, ciprofloxacin, linezolid, clindamycin shows good sensitivity against isolated bacterial species. Conclusion- this study concludes that in vitro testing previous to antibiotic use may help in the prevention and treatment of multi-drug resistant pathogens in burn infection. Isolation pattern and antibiogram of burn wound of this study provides adequate and effective treatment the will decrease the rate of morbidity and mortality and other systemic antibiotic policy will be used for burn patients.

Keyword-Antibiotic susceptibility pattern, Antibiogram, Biochemical test, indiscriminate, mortality and morbidity, Wound infection, systemic antibiotic policy,

Introduction

Nosocomial infection is a major cause of morbidity and mortality in burn patients, in Infections are an important cause of morbidity and mortality in patients with burns. Wound infections are one of the most common sites of nosocomial infections in burn patients with a prevalence of about 60%, followed by bloodstream infections (20%), urinary tract infections (20%) and pneumonia (10%) [1]. Burn wound infections can lead to scarring, bacteremia, sepsis, and multi-organ dysfunction, contributing to 75% mortality in burn patients [2,3]. The occurrence of nosocomial burn infections depends on several factors such as the burn severity, immune status, prolonged stay, invasive procedures and overcrowding leading to cross infections [4]. Burn units are often the sites of

major and prolonged outbreaks with resistant organisms [5]. The rate of nosocomial infections is higher in burn patients due to various factors like nature of burn injury itself, the immune compromised status of the patient, invasive diagnostic and therapeutic procedures and prolonged ICU stay.[6].

HAI usually associated with invasive procedures through medical devices or surgical procedures [7,8], *Staphylococcus aureus, Acinetobacter baumannii and Pseudomonas aeruginosa* were the most common nosocomial pathogens in their burns center. The infecting microorganism may belong to aerobic as Most commonly isolated aerobic microorganism include *Staphylococcus aureus*, Coagulase-negative *staphylococci* (CoNS), *Enterococci, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter species, Proteus mirabilis, Candida albicans and Acinetobacter* [9,10].

Wound infections have been a problem is the field of medicine for a long time. The risk of serious infection even with relatively small bacterial inoculums[11]. Advances in control of infections have not completely eradicated this problem because of the development of drug resistance [12]. The widespread uses of antibiotics, together with the length of time over which they have been available have led to major problems of resistant organisms contributing to morbidity and mortality [13,14,15]. Antimicrobial resistance can increase complications and costs associated with procedures and treatment [16]. Knowledge of the causative agents of wound infection in a specific geographic region will, therefore, be useful in the selection of antimicrobials for empiric therapy. This study was carried out to determine the antibacterial susceptibility of bacteria isolated from wound infections as well as update the clinicians in the various antimicrobial alternatives available in the treatment of wound infections.

The number of antibiotic-resistant bacteria has increased in recent years and such resistance can compromise the efficacy of antimicrobial therapy. Antibiotic-resistant bacteria can be associated with infection with higher mortality than those caused by antibiotic susceptibility strain [17].

The therapy of both nosocomial and community-acquired infection is affected by the continuing evolution of and challenges presented by antimicrobial resistance. This increasing emergence and spread of multidrug-resistant bacteria in hospital in general and burn centers, in particular is of great concern and continues to challenge infection control and hospital epidemiology practice worldwide [18]. Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is due to the extensive use and misuse of antimicrobial drugs which have favored the emergence and survival of resistant strains of microorganisms [19].

On a study in Iran on the bacterial infection of burn patients at a burn hospital, the microorganism causing infections were *Pseudomonas aeruginosa* (37.5%), *Staphylococcus aureus* (20.2%), and *Acinetobacter baumanni* (10.4%). Among these isolates *Pseudomonas aeruginosa* was found to be 100 percent resistant to amikacin, gentamycin, carbenicillin, ciprofloxacin, tobramycin, and ceftazidime; 58% of S aureus and 60% of coagulase-negative *Staphylococcus* were methicillin resistant. Multidrug-resistant bacteria have frequently been reported as the cause of nosocomial outbreaks of infection in burn units or as colonizers of the wounds of the burn patient. Similarly, antimicrobial resistance in some of the most frequent bacterial species isolated from burn patients includes *Stayphylococcus aureus, Pseudomonas aeruginosa*, and other gram-negative bacilli has reached a worrying level [1,20].

The aim of the present study to determine the pattern and extent to surveillance of nosocomial infection in burn wound patients and their antimicrobial susceptibility effect on burn patients and reduce the rate of death of patients in MBS hospital, Kota.

MATERIAL AND METHOD

A. Sample collection:

Sampling from burn ward :

For identified the nosocomial infection in burn patient the air samples were collected using sterile swabs from all areas of MBS Hospital Kota burn ward, hydrotherapy room, dressing room and equipment of burn ward during 15 day period and to rule out the possible transmission from environment to the patients.

Sample collection from patient:-

A swab from the patient burn wound was collected after cleansing it with sterile normal saline. Effective serous or pus discharge is collected in two swabs. First pus swab was used for presumptive diagnosis with staining and second swab is used for culture isolation.

All samples were labeled properly and immediately transported to the Microbiology laboratory. Swab taken from different sites was inoculated on Nutrient agar, Blood agar and MacConkey agar. These culture plates were incubated at 37°C under aerobic condition for 24 hours. Isolation and identification of isolates is done as per standard guidelines.

B. Identification of isolates organism:-

All the samples were cultured on Blood agar, Mac-Conkey agar and Nutrient agar plates and was incubated overnight at 37°C, depending upon the organism suspected they was subcultured on various selective media. The bacteria were further identified by colony morphology, Gram's staining and conventional biochemicals tests.

C Antimicrobial susceptibility testing

Kirby- Bauer disc diffusion technique was performed to evaluate the antimicrobial susceptibility pattern of isolates. A suspension of each isolate was made so that the turbidity becomes equal to 0.5 McFarland standards and they plated onto- Muller-Hinton agar plate (Himedia). Sterile commercially available antibiotics filter paper disc onto which a definite amount of antibiotic has been absorbed were applied to each plate. After incubation at 37^oC for 16-18 hours, zone size was measured.

Procedure:

The suspension of the test organisms was prepared by picking four to five well- isolated colonies of bacteria with similar morphology from Nutrient agar plate with a sterile wire loop. These were suspended in nutrient broth and incubated at 37°C for 2-4 hrs. The density of suspension to be inoculated was determined by comparison with opacity standard on McFarland 0.5. A sterile swab dipped into the suspension of inoculums and removed excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of liquid and then spread over the agar plate so as to get a matt growth. Sterile antibiotics discs were equidistantly placed (not closer than 24 mm center) to these plates and gently pressed onto the medium with the help of sterile forceps to ensure complete contact with the help of then incubated at 37°C for 16-18 hours. A zone of inhibition was measured in millimeters and the organisms classified as sensitive, intermediate or resistant according to the zone size interpretation chart.

RESULT:-

We analyzed total 100 samples, 45(45%) from female and 55(55%) from male patients were processed during this study period from Jan 2016 to Dec 2016 at the microbiology laboratory of MBS central lab Kota, Rajasthan.

The present study Out of 100 samples, *Pseudomonas spp.* was the most common organism that is 37 (37%) in our study. Followed by *Escherichia coli* that is 26%, *Klebsiella spp.* 14%, *Staphylococcus aureus* 12 *%, Acinetobacter* 8%, *Enterobacter* 2% and *proteus* 1%.(table - 1).

Table:- 1: organisms wise distribution of culture sample

Organisms	No of isolates	Percentage
Pseudomonas	37	37%
E.coli	26	26%
Klebsiella	14	14%

57

58

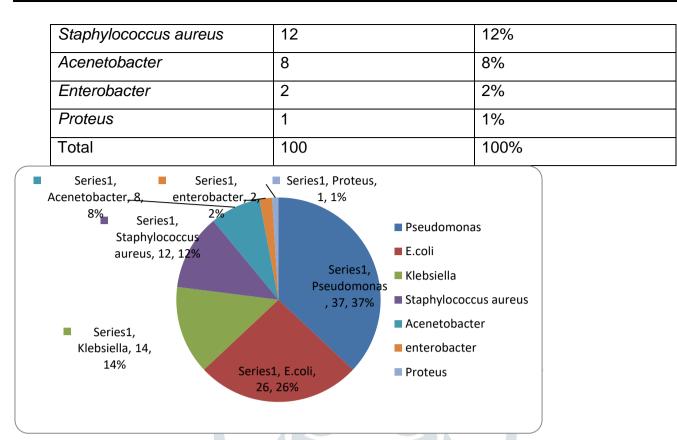


Figure 1 – Organisms wise distribution of culture sample

Pseudomonas spp. was most susceptible to the colistin (83.78%), ciprofloxacin (67.56%) , tazobactum+piperacillin (51.35%) ,Moxifloxacin (45.94%), Levofloxacin (45.94%), Imipenem (45.94%), Cefoperazon+sulbactum (40.54%), Amikacin (40.54), Carbenicillin (37.83) and meropenem (32.43%). These isolates were least susceptible to Azithromycin (27.02%), ceftriaxone (18.91%), Ampicillin/sulbactam (16.21%), Co-trimoxazole (13.51),cefuroxime (8.10%), Ceftazidime (8.10%), cefepime (8.10%), cefotaxime (5.40%), clarithromycin (5.40%), Cefpodoxime ,cefaclor, Amoxyclav+cefuroxime and ampicillin (2.70%).(table -2). Amikacin a second generation amino glycoside was effective against *Pseudomonas, E.coli*, and *Klebsiella* in our study [21,22]. In a study by Agnihotri N, Gupta V, Joshi RM, *Pseudomonas* was the commonest isolate in the burn wounds and amikacin was found to be the most effective drug against gram-negative bacteria [21].

Table:-2 Antibiotic susceptibility pattern of Pseudomonas species.

Sr.	Antibiotics (drugs)	Pseudomonas (n=37)				
No.	(25 drugs)	No of susceptible	%			
		isolates				
1.	Clarithoromycin	2	5.40%			

2.	Ciprofloxacin	25	67.56%
3.	tazobactum+piperacillin	19	51.35%
4.	Meropenem	12	32.43%
5.	Augmentin	-	
6.	Co-trimoxazole	5	13.51%
7.	Linezolid	-	
8.	Ampicillin	1	2.70%
9.	Ampricillin/sulbactum	6	16.216%
10.	amoxyclav+cefuroxime	1	2.70%
11.	Colistin	31	83.783%
12.	Moxifloxacin	17	45.945%
13.	cefoperazon+sulbactum	15	40.540%
14.	Amikacin	15	40.540%
15.	Cefepime	3	8.108%
16.	Levofloxacin	17	45.94%
17.	Ceftnaxome	7	18.91%
18.	Carbenicillin	14	37.83%
19.	Imipenem	17	45.945%
20.	Azithromycin	10	27.02%
21.	Ceftazidime	3	8.108%
22.	Cefaclor	1	2.70%
23.	Cefuroxime	3	8.108%
24.	Cefodoxime	1	2.70%
25.	Cefotaxime	2	5.40%

In our study, isolates of *Pseudomonas aeruginosa* from wound swab and pus were found relatively more resistant than those from other specimens. The rate of resistance (%R) for Colistin was 83.78%, Ciprofloxacin was 67.56%, Tazobactum + piperacillin was 51.35%, Moxifloxacin, imipenem, and Levofloxacin was 45.94, Amikacin and cefoperazon+Sulbactum was 40.54%, Carbenicillin was 37.83%, Meropenem was 32.43%, Ceftriaxone was 18.91%. the previous study in 2000-2001, in Bangladesh, showed that %R of P. aeruginosa to co-trimoxazole was 92%, ciprofloxacin 62.5%, cephalexin 100%, ceftriaxone 75%, and ceftazidime 37%.7 However, in another study, the resistance to amikacin was 2%, ceftriaxone 43%, ceftazidime 25% and ciprofloxacin 50%.The number of multi-drug resistant strains has increased in recent years. An

Iranian study in 2003 had their resistance pattern worse than this study, where the %R to gentamycin was 93.7%, ceftazidime 96%, amikacin 93%, and ciprofloxacin 86%. A five-year retrospective Indian study, from 1997-2002, found resistance pattern of *Pseudomonas aeruginosa* as amikacin 52%, gentamycin 69%, ciprofloxacin 89%, and ceftazidime 62%, which shows that the trend of resistance was also increasing. Taneja [23] found the pattern of resistance about 90% each for ceftazidime and amikacin while 45 % for ciprofloxacin.

Staphylococcus aureus showed maximum susceptibility to the 3 drugs Linezolid (83.33%), Ciprofloxacin (83.33%) and Gentamycin (83.33%) followed by Clindamycin (75%), Vancomycin (75%), Cefrizoxime (58.33%), ceftrianxone (58.33%), Cefoperazon+sulbactum (41.66) and cefpodoxime (41.66%). Penicillin, cefotaxime, azithromycin, moxifloxacin, cefuroxime, ampicilin+sulbactum, cefotetan, meropenem, cefixime, cefazolin, amikacin, Co-trimoxazole, levofloxacin and imipenem were the drugs to which *Staphylococcus aureus* isolates were least susceptible 8.33%, 33.33%, 25%, 25%, 33.33%, 16.66%, 8.33%, 25%, 8.33% respectively.(table 3). Antibiotic sensitivity patterns revealed that many of the isolates were resistant to commonly used antibiotics like cephalosporin group, quinolones, etc. which are being indiscriminately used an an empirical basis for a a prolonged duration of time. Resistance to various antibiotics routinely used has been reported from several studies. *Staphylococcus aureus* and *Staphylococcus epidermidis* were seen to be sensitive to Amikacin and Piperacillin-tazobactam [21].

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Sr.	Antibiotics (drugs)	Staphylococcus aureus						
No.		(n=12)						
		No . of						
		susceptible	%					
		isolates						
1.	Penicillin	1	8.33%					
2.	Cefotaxime	3	25%					
3	Azithromycin	4	33.33%					
4.	Clindamycin	9	75%					
5.	Vancomycin	9	75%					
6.	Linezolid	10	83.33%					
7.	Moxifloxacin	3	25%					
8.	Cefoperazon+ sulbactum	5	41.66%					
9.	Cefuroxime	3	25%					

Table:- 3: antibiotic susceptibility pattern of *Staphylococcus aureus*

10.	Ciprofloxacin	10	83.33%
11.	Cefopdoxime	5	41.66%
12.	Gentamycin	10	83.33%
13.	Ampicilin+sulbactum	4	33.33%
14.	Amoxyclav	2	16.66%
15.	Ceftizoxime	7	58.33%
16.	Ceftnaxone	7	58.33%
17.	Cefotetan	1	8.33%
18.	Meropenem	3	25%
19.	cefixime	1	8.33%
20.	Cefazolin	1	8.33%
21.	Amikacin	1	8.33%
22.	co-trimoxazole	2	16.66%
23.	Levofloxacin	3	25%
24.	Imipenum	1	8.33%

E. coli isolates was most sensitive to the amikacin (80.76%), (Meropenem(69.23%), Gentamycin, Imipenem (57.69%), Cefoperazon+ sulbactum (46.15%), Colistin (38.88%), Tazobactum+piperacillin (34.61%) and Levofloxacin (26.92%).these isolates were least susceptible to moxifloxacin, ampicillin/sulbactam, Nitrofurantoin (19.23%), Ciprofloxacin, Azithromycin, Cefpodoxime (15.38%), Ceftriaxone, ceftazidime clav, cefotxime+clav (7.69%), Cefuroxime and Cefotaxime (3.84%). Sensitivity pattern of E.coli compared to other studies were ciprofloxacin (97%), cefazolin (92%) [24], ceftazidime (91%), ofloxacin (97%) [25]. So reduce antibiotic sensitivity pattern noted for E.coli suggests its important for nosocomial infection. In other studies, 100% of E.coli isolates were resistant to ampicillin, cefaclor, doxycycline and amoxicillin, 87.5% to erythromycin, cefuroxime, cefotaxime, and cefazolin [26].

Klebsiella spp. was most sensitive to the colistin (78.57%), amoxicillin+clavulanic acid, Gentamycin (57.14%), Meropenem (50%) and Ciprofloxacin (42.85%). These isolates were least susceptible to Azithromycin, Cefotxime+clav (35.71%), Amikacin, Imipenem, Ampicillin/sulbactum, Cefoperazon+sulbactum (28.27%), Ampicillin, Cefuroxime, Levofloxime, tazobactum+piperacillin, ceftazidime clav, cefotaxime (21.42%), Nitrofurantoin and cefpodoxime (12.5%). As compared to other studies Klebsiella spp was sensitive to amikacin, ceftazidime, cefotaxime(100%)(Manikandan et al., 2013)[26], however to the the previous study had shown reduced sensitivity to ciprofloxacin (63%), cefazolin (44.7%), ceftazidime (36.8%), and cefuroxime (34.2%) [27].

Acinetobacter spp. isolates were most susceptible to the Gentamycin, amikacin, Tazobactum+Piperacillin, Cefoperazon+sulbactum (87.5%), ciprofloxacin, levofloxacin, ampicillin/sulbactam, Moxifloxacin (62.5%), Imipenem, azithromycin (50%). Meropenem, Cefotxime+clav (37.5%). These isolates were least susceptible to Cefuroxime, Amoxicillin +clavulanic acid (25%), Ampicillin, Cefepime, Nitrofurantoin, Ceftriaxone, Ceftazidime clav, Cefpodoxime and Cefotaxime (12.5%). In other studies Enterobacter spp. was sensitive to amikacin(80%), Tazobactum+Piperacillin (50%), levo-ofloxacin (20%), colistin (100%),

In this study *Proteus spp.* isolates were more susceptible to Ampicillin, Gentamycin, Amikacin, Imipenem, Ciprofloxacin, Meropenem, Ceftriaxone and Ceftazidime clav (100%) these isolates were non-susceptible to Cefuroxime, Levofloxacin, Nitrofurantoin, Clarithromycin, Tazobactum+piperacillin, Co-trimoxazole and Cefpodoxime(0%). As compared to other *proteus spp.* was sensitive to amikacin (100%), gentamycin, ceftazidime, ofloxacin (90%), ciprofloxacin (79.5%) and tobramycin (79.5%). (Manikandan et al., 2013)[25], as compared to previous studies the sensitivity rate were reduced for ciprofloxacin (75%), cefazolin (37.50%), ceftazidime (37.50%), cefuroxime (25%), and ampicillin (95%) [27].

Enterobacter spp. were highly susceptible to the Gentamycin, Amikacin and Moxifloxacin (100%). These isolates were least susceptible to Ampicillin, Ciprofloxacin, Tazobactum+piperacillin, Meropenem, Moxifloxacin and Clarithromycin(50%).

Table:-4 Antibiotic susceptibility pattern of Enterobacteriaceae family- Klebsiella spp., Acinetobacter spp., E.coli spp., Proteus spp., Enterobacter spp.

	Antibiotics	E.coli		Klebsiella		Acinetobacter		Proteus		Enterobacter	
Sr.	(26 drugs)	n =26		n = 14		n =8		n =1		n =2	
no.		No	%	No	%	No	%	No	%	No	%
1	Ampicillin	2	7.69	3	21.32	1	12.5	1	100	1	50
2	Cefepime	1	3.84	00	00	1	12.5	00	00	00	00
3	Amoxicillin +clavulanic	1	3.84	8	57.14	2	25	00	00	00	00
	acid										
4	Gentamycin	15	57.69	8	57.14	7	87.5	1	100	2	100
5	Amikacin	21	80.76	4	28.57	7	87.5	1	100	2	100
6	Imipenem	15	57.69	4	28.57	4	50	1	100	00	00
7	Ciprofloxacin	4	15.38	6	42.85	5	62.5	1	100	1	50
8	Cefuroxime	1	3.84	3	21.42	2	25	00	00	00	00

9	Levofloxscin	7	26.92	3	21.42	5	62.5	00	00	-	-
10	Nitrofurantion	5	19.23	1	7.14	1	12.5	00	00	00	00
11	Clarithoromycin	00	00	-	-	-	-	00	00	1	50
12	Tazobactum+piperacillin	9	34.61	3	21.42	7	87.5	00	00	1	50
13	Meropenem	18	69.23	7	50	3	37.5	1	100	1	50
14	Co-trimoxazole	3	11.53	2	14.28			00	00	00	00
15	Ampicillin/sulbobactum	5	19.23	4	28.57	5	62.5	-	-	-	-
16	Colistin	14	38.88	11	78.57	6	75	-	-	2	100
17	Moxifloxacin	5	19.23	2	14.28	5	62.5	-	-	1	50
18	Cefoperazon+sulbactum	12	46.15	4	28.27	7	87.5	-	-	-	-
19	Ceftriaxone	2	7.69	2	14.28	1	12.5	1	100	-	-
20	Ceftazidime clav	2	7.69	3	21.32	1	12.5	1	100	00	00
21	Azithromycin	4	15.38	5	35.71	4	50	-	-	00	00
22	Cefotxime+clav	2	7.69	5	35.71	3	37.5	-	-	-	-
23	Cefpodoxime	4	15.38	1	7.14	1	12.5	00	00	00	00
24	Cefotaxime	1	3.84	3	21.42	13	12.5	-	-	-	-

A high %R to antibiotics indicates the improper use of antibiotics in the hospital [28]. The result of this study indicates that commonly used drugs can no more be used as empirical therapy for suspected *pseudomonad* infections. We clearly indicate that wound infection is the most common cause of nosocomial infection. Similarly, Lari *et al* at Tohid Burn Center in Tehran, reported wound infection to be the main cause of nosocomial infection [29].

There is an alarming increase of infections cause by antibiotic resistant bacteria. Lack of uniform antibiotic policy and indiscriminate use of antibiotics may have lead to emergence of resistant bacterial strain. In our study Amikacin, meropenom, gentamycin, imipenem, ciprofloxacin,I inezolid, clindamycin shows good sensitivity against isolated bacterial species. According to recent classification systems infection control programs need to file and report burn wound infection. Surveillance for surgical site infections and reporting of these rates to surgeons has been shown to reduce the rates of infection [30]. The infection control literature indicates that precise, written definitions are essential to accurately identify hospital-acquired infections. It has been suggested that because of discrepancies between the surgeon's assessment and infection control assessment, burn patients are over-treated with antimicrobial agents and antimicrobial use could possibly be decreased if more precise definitions of infection were used in clinical practice

[31]. Decreased use of invasive devices, and improved aseptic technique when inserting devices could decrease the rates of nosocomial infections in burn units.

CONCLUSION

This study concludes that in vitro testing previous to antibiotic use may help in the prevention and treatment of multi-drug resistant pathogens in burn infection. Isolation pattern and antibiogram of burn wound of this study provides adequate and effective treatment the will decrease the rate of morbidity and mortality and other systemic antibiotic policy will be used for burn patients.

To hospital antibiotic policy, the high percentage of multidrug-resistant isolate is probably due to the experiential use of wide-ranging antibiotics and non-observance. The early finding of isolates is also very important to prevent treatment failure as the time involved in isolation, identification and performing antibiotic sensitivity can take as long as 48 hours from the receipt of the specimen. This time period may be enough to allow a subclinical infection to become life threating illness, secondly, in burn wound, because of the mixed infection, the potential virulence of one organism may affect another organism growing alongside. one more factor adding to the problem is multidrug resistance (MDR) of the organism.

This study would be useful to describe the antimicrobial procedure of the hospital. Microbiological observation should be the ongoing process to determine change in colonizing bacteria. According to the study we suggest to MBS hospital, Kota,the following criteria to decrease the rate of nosocmial infection in burn patients and decrease the rate of morbidity and mortility in MBS hospital:-

• To prevent the spread infection within burned patients; the implementation of contact precautions (single use masks, gowns, and gloves are damaged while in contact with the patient and the hands are washed after finishing contact with the patient, cohort nursing (grouping patients of a given colonization status, with designated Health Care Workers, and a targeted minimum ratio of 1:1 of nursing staff to patients), strict adherence to aseptic techniques for changing dressings, hand disinfection and location of hand disinfectant (alcohol 70% isopropanol/ethanol) dispensers near all beds and installation of Laminar airflow techniques in burn units.

• Systemic antibiotics use for burn patients.

• In addition, regular antimicrobial susceptibility observation is important for ward wise supervision of the resistance pattern.

• For better patient physical condition and health management a effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics.

• The overfilling in burns ward is an important cause of major infection and be required to be avoided in order to control a nosocomial infection.

• Before the start of antibiotic therapy and restrictive antibiotic policy, MDR strains become conventional in the hospital atmosphere these can continue for months. as a result, and thus decrease overall infection-related morbidity and mortality careful microbiological surveillance and in vitro testing may be of great help in prevention and treatment of MDR isolates in burn wards.

ACKNOWLEDGEMENT

In this study was supported by the central lab (microbiology) department of New Medical college and MBS hospital Kota. and especially thankful to Dr. Naveen Saxena(Medical superintendent in MBS hospital and Kota medical college.) for their support.

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