AEROBIC BACTERIAL ISOLATES IN SUPPURATIVE INFECTIONS AND THEIR ANTIBIOGRAMS - A REFLECTION OF INFECTION CONTROL

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ABSTRACT

Aims: Wound infections have been a serious concern for the healthcare practitioners worldwide. The present study aims at isolation of bacterial pathogens from suppurative wound infections along with their antibiograms to evaluate the prevailing infection control strategies which shall aid in further successful wound management. *Materials and Methods:* Study was conducted on 322 pus samples collected from patients admitted to various clinical departments at Katuri Medical College & Hospital, Guntur (A.P), India, to analyze the distribution of aerobic bacterial pathogens & their antimicrobial susceptibility pattern. *Results:* Out of 322 samples, 288 (89.45%) were culture positive & 34 (10.55%) remained sterile. Gram negative isolates had a predominance of Pseudomonas aeruginosa 94 (24.22%), whereas in Gram positive isolates, Staph. aureus 86 (22.16%) remained commonest .Staph aureus remained susceptible to Chloramphenicol (91%), Gatifloxacin (85%), Vancomycin (80%) and Oxacillin (50%). Gram negative, oxidase positive isolates showed high resistance for third generation cephalosporins i.e. Cefopodoxime (88%), Cefotaxime (54%), and Ceftazidime (45%) except, Ceftriaxone to which none of the isolate showed resistance but remained 100% sensitive to Imipenem, Meropenem and 90% sensitive to Piperacillin/Tazobactam. Gram negative, oxidase negative isolates depicted highest sensitivity to Imipenem (100%), Piperacillin/Tazobactam (95%), followed by Amikacin (63%). *Conclusion:* As high proportion of samples depicted positive growth, strict infection control is recommended as a strategy to minimize spread of resistant organisms. Further studies need to be extended to include cultures under anaerobic conditions and also to establish the presence of other organisms which require such environment for their growth.

KEY WORDS

Antibiogram, Infections, Suppurative, Wounds.

INTRODUCTION

A wound is a breach in the skin & the exposure of subcutaneous tissue following loss of integrity provides a moist, warm, nutritive environment that is conducive to microbial colonization & proliferation[1]. In spite of technological advances that have been made in surgery & wound management, wound infection has been regarded as the most common nosocomial infection especially in patients undergoing surgery[2]. In recent years, skin & soft tissue infections (SSTI's) particularly to multidrug resistant pathogens is increasingly being encountered in clinical settings. SSTI's may range from simple uncomplicated & superficial infections such as

folliculitis, cellulitis, and abscesses to deeper complicated infections such necrotizing fasciitis & diabetic foot[3]. The knowledge of the causative agents of wound infection has therefore proved to be helpful in the selection of empiric antimicrobial therapy & on infection control measures in health institutions[4].

MATERIALS AND METHODS

An analytical cross sectional study of 322 cases was done in the Department of Microbiology, Katuri Medical College & Hospital, Guntur (A.P.) India from June 2010 to August 2010 & so designed, to analyze the distribution of bacterial pathogens isolated from pus samples & their antimicrobial susceptibility pattern.

Sample collection:

Pus samples were collected aseptically from surgical sites, necrotizing soft tissues, non- healing ulcers, diabetic foot, before the local area was cleaned by an antiseptic solution. The samples were collected by sterile cotton tipped swabs & direct aspiration of pus from deep seated wounds. The samples were transported to the laboratory within 30 minutes of collection. In the laboratory, samples were registered & macroscopically examined for their appearances. The swabs were cultured & smears made on clean slides for Gram staining techniques.

Microscopic examination & Culture:

Smears were air dried & heat fixed .Staining was done by routine Gram's technique which aids in grouping the bacterial pathogens into Gram positive or Gram negative. The specimens were inoculated on both differential & enriched media (MaConkey agar & Blood agar respectively).The culture plates were kept for aerobic incubation for 24-48 hours before colonial morphologies were studied & interpreted.[5]

Identification of bacterial pathogens:

Preliminary identification of bacteria was based on colony characteristics of the organisms i.e. haemolysis on Blood agar, changes in physical appearance in differential media & enzyme activities of the organisms. Biochemical tests were performed on colonies from primary cultures for final identification of the isolates.[6]

Antibiotic susceptibility testing:

Susceptibility testing was performed by Kirby-Bauer technique ,using commercially available antibiotic impregnated paper discs (Hi-Media).[7] The plates were incubated at 37°C for 16-18 hours as recommended by Clinical Lab Standards Institute.[8] After incubation, the clear zones around the discs were measured with a ruler & recorded in millimeter scale.[9] Drugs tested include Ampicillin (10μg), Amoxycillin (25μg), Chloramphenicol (30μg), Cotrimoxazole (25μg), Erythromycin (15μg), Oxacillin (1μg), Vancomycin (30μg), Ofloxacin (5μg), Gatifloxacin (5μg), Sparfloxacin (5μg), Ciprofloxacin (5μg), Gentamicin (10μg), Amikacin (30μg), Tobramicin (10μg), Cefazolin (30μg), Cefoperazone (75μg), Cefotaxime (30μg), Ceftazidime (30μg), Ceftriaxone (30μg), Cefopodoxime (10μg), Aztreonam (30μg), Piperacillin / Tazobactam (100/10μg), Imipenem (10μg) and Meropenem (10μg).

RESULTS

During the study period, 322 pus samples were collected from different specialties for culture and sensitivity, out of which 288 (89.44%) were culture positive & in 34 (10.55%) no bacterial isolate was obtained. Out of the 288 culture positive samples, only 4 (1.39%) had fungal growth while the remaining 284 (98.61%) showed bacterial growth.

Out of 288 culture positive samples, 388 isolates were obtained. Out of 388 isolates, 196 (50.52%) had pure growth, whereas cultures with two isolates were 156 (40.20%) and polymicrobial growth was seen in 36 (9.28%).Majority of the pus samples received were from general surgery 170 (52.79%), followed by orthopedics department 80 (24.89%) as shown in **Table 1**. Samples received from OBG / Gynae Casualty, General medicine were 18 (5.59%), 18 (5.59%) & 14 (4.34%) respectively. Out of 388 isolates, Pseudomonas aeruginosa topped the list 24.22% followed by Staphylococcus aureus 22.16% as shown in **Table 2**. Staph. aureus remained 100% resistant to Sparfloxacin and Ceftazidime followed by Ampicillin (95%) & Amoxycillin (86%).Highest sensitivity was reflected to Chloramphenicol (91%) followed by Gatifloxacin (85%) and Vancomycin (80%) as shown in **Table 3**. Gram negative, oxidase negative isolates

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depicted 100% resistance to Ampicillin & Amoxycillin followed by Cefopodoxime (92%) and Cefazolin (82%). Among Fluoroquinolones, highest resistance was shown to Ciprofloxacin (73%) followed by Sparfloxacin (71%), Ofloxacin (69%), and Gatifloxacin (45%). All the isolates remained 100% sensitive to Imipenem as shown in **Table 4**. All Gram negative; oxidase positive isolates remained 100% susceptible to Imipenem, Meropenem, Ceftriaxone and Aztreonam as shown in **Table 5**.

Table 1: Specimens and their distribution from various departments

Departments	Number of samples	Percentage	Culture positive samples	Percentage
General surgery	170	52.79	158	54.86
Orthopedics	80	24.89	72	25.00
OBG/Gynae	18	5.59	16	5.55
Casualty	18	5.59	16	5.55
General Medicine	14	4.34	10	3.47
Dermatology	10	3.10	6	2.08
Pediatrics	6	1.86	4	1.38
ENT	4	1.24	4	1.38
Ophthalmology	2	0.62	2	0.69
Total	322	100.00	288	100.00

Table 2: Characterization of organisms isolated

Isolate	Frequency	Percentages
Pseudomonas aeruginosa	94	24.22
Staphylococcus aureus	86	22.16
E. coli	68	17.52
Klebsiella pneumoniae	36	9.27
Proteus spp	30	7.73
Enterococcus spp	20	5.15
Enterobacter spp	14	3.60
Citrobacter spp	10	2.57
Providencia spp	10	2.57
Acinetobacter spp	6	1.54
Beta-hemolytic streptococci	6	1.54
Candida spp	4	1.03
Edwardsiella spp	2	0.51
Serratia marcescens	2	0.51
Total	388	100.00

Table 3: Staphylococcus aureus antibiogram

Antibiotic	Resistant(%age)	Sensitive (%age)
Ampicillin	95	5
Amoxycillin	86	14
Cotrimoxazole	77	23
Chloramphenicol	9	91
Erythromycin	52	48
Vancomycin	20	80
Oxacillin	50	50
Ofloxacin	71	29
Gatifloxacin	15	85
Sparfloxacin	100	0
Ciprofloxacin	74	26
Gentamicin	44	56
Amikacin	50	50
Cefoperazone	63	37
Cefazolin	71	29
Cefotaxime	71	29
Ceftazidime	100	0
Piperacillin/Tazobactam	84	16

Table 4: Gram negative, oxidase negative antibiogram

Antibiotic	Resistant (%age)	Sensitive (%age)
Ampicillin	100	0
Amoxycillin	100	0
Cotrimoxazole	74	26
Chloramphenicol	28	72
Ofloxacin	69	31
Ciprofloxacin	73	27
Sparfloxacin	71	29
Gatifloxacin	45	55
Gentamicin	64	36
Amikacin	37	63
Tobramicin	74	26
Cefazolin	82	18
Cefoperazone	66	34
Cefotaxime	72	28
Ceftazidime	71	29
Cefopodoxime	92	8
Imipenem	0	100
Piperacillin/Tazobactam	5	95

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Table 5: Gram negative, oxidase positive antibiogram

Antibiotic	Resistance (%age)	Sensitive (%age)
Ampicillin	100	0
Co-trimoxazole	65	35
Ciprofloxacin	74	26
Ofloxacin	61	39
Gatifloxacin	18	82
Gentamicin	12	88
Amikacin	11	89
Cefazolin	56	44
Cefoperazone	50	50
Cefotaxime	54	46
Ceftazidime	45	55
Cefopodoxime	88	12
Ceftriaxone	0	100
Aztreonam	0	100
Piperacillin/Tazobactam	10	90
Imipenem	0	100
Meropenem	0	100

DISCUSSION

The development of wound infection depends on the integrity and protective function of the skin.[10] It has been shown that wound infection is universal and the bacterial type varies with geographical location, resident flora of the skin, clothing at the site of wound, time between wound and examination.[11] In the present study, positive growth was observed in 89.45% of wound cultures which is similar to isolation rate of 95% in a study done in Nigeria by Shittu et al,2002.[4] The incidence of Pseudomonas aeruginosa in post operative wound infection is becoming more serious in developing countries because of relaxation in general hygienic measures, mass production of low quality antiseptic and medicinal solutions for treatment, difficulties in proper definition of the responsibility among the hospital staff.[12] Our results show that frequency of Pseudomonas aeruginosa was 24.22% of all the pathogens isolated from wound infections, which is in concordance with Motayo et al, 2013 and Masaadeh and Jaran,2009 where the frequency was 25.4% and 27.8% respectively, but, lower than the study done in Africa by Oguntibeju and Nwobu,2004 as well as a study done in India by Revathi et al,1998 who reported 33% and 36% respectively.[11,13,14,15] Staphylococcus aureus was the second most common isolate 22.16% which is similar to study done in Nigeria by Shittu et al,2002 who also reported Staph. aureus 25.3%.[4] Nasal carriage of *S. aureus* has been identified as an important risk factor for the acquisition of *S. aureus* infection, although this may depend on an array of factors that may either be environmental or patient-related.[16] The postulated sequence of events which lead to infection is initiated with S.aureus nasal carriage which is disseminated via hand carriage to other body sites where infection can occur with breaks in dermal surfaces.[4] Staphylococcus aureus was found to be susceptible to Chloramphenicol (91%), Gatifloxacin (85%), Vancomycin (80%) and Oxacillin (50%). Resistant pattern of S.aureus for fluoroquinolones was Sparfloxacin (100%), Ciprofloxacin (74%), and Ofloxacin (71%). Our study has shown reduced sensitivity to Gentamicin (56%) and Amikacin (50%). However most of the isolates, both gram negative and gram positive were resistant to Ampicillin, Amoxicillin, Co-trimoxazole and Ciprofloxacin.This resistance may be due to the reason that these antibiotics have been in use for a long time both therapeutically as well as for prophylactic use and secondly their oral route of administration may effect their rate of absorption as well as their therapeutic

efficacy. Gram negative, oxidase negative isolates have shown highest sensitivity to Imipenem (100%), Piperacillin/Tazobactam (95%), followed by Amikacin (63%). The possible reason can be lesser usage of these agents; moreover antibiotic susceptibility pattern varies from place to place. Third generation cephalosporins depicted resistance pattern as Cefopodoxime (92%), Cefotaxime (72%), and Ceftazidime (71%).Gram negative, oxidase positive isolates showed high resistance for third generation cephalosporins i.e. Cefopodoxime (88%), Cefotaxime (54%), and Ceftazidime (45%) except, Ceftriaxone to which none of the isolate showed resistance. This means that Ceftriaxone has not been misused as other cephalosporins; hence it is highly effective as compared to those antibiotics that have been in use for a longer time. Among fluoroquinolones, highest sensitivity was shown to Gatifloxacin (82%) as compared to Ciprofloxacin (26%) and Ofloxacin (39%). This pattern may be due to indiscriminate usage of fluoroquinolones as prophylactic agents except for Gatifloxacin. All the isolates remained 100% sensitive to Imipenem and Meropenem and 90% sensitive to Piperacillin/Tazobactam which is similar to the study done by Mahmood, 2000 who reported sensitivity to Imipenem as 96% and Piperacillin/Tazobactam as 89%..[17] Farida and Mir, 2010 also reported sensitivity to Meropenem as 97%.[18]

SUMMARY AND CONCLUSION

As high proportion of samples depicted positive growth, infection control is recommended as a strategy to minimize spread of resistant organisms. In this study, variety of organisms were isolated which support the need to obtain specimens from infected wounds for microbiological evaluation as well as antibiotic susceptibility testing, which shall facilitate successful wound management .This study reflected Vancomycin to be the most effective antibiotic against gram positive organisms. This study also showed that Imipenem was highly effective against Gram negative, oxidase positive organisms while Ceftriaxone, Aztreonam, Imipenem and Meropenem were highly effective against Gram negative, oxidase positive organisms. The knowledge of susceptibility testing patterns of the bacterial strains will guide the clinicians to choose appropriate and judicious antibiotics for treatment of wound infections. Updating the antibiogram will further reduce the complications of resistance. Moreover there is need to develop national surveillance of antibiotic-resistant organisms.

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