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Original Research Article

A Study on Bacterial Pathogens in Wound Infections at Ganni Subba Lakshmi Medical College

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ABSTRACT

The objective of present study was to analyze the pathogenic bacteria and its antimicrobial profile of wound infections, carried out in the department of Microbiology, GSL Medical College, Rajahmundry, Andhra Pradesh from May 2011 to May 2013. Out of a total 500 samples studied 425 bacterial isolates were found. The results showed that the presence of gram positive bacteria like Staphylococcus aureus (24%), Staphylococcus epidermidis (14.82%) and gram negative bacteria like Escherichia coli (19.76%), Pseudomonas aeruginosa (18.82%), Klebsiella pneumoniae (15.52%) and Proteus vulgaris (7.05%). Staphylococcus aureus was most predominant bacteria in wound infection followed by Escherichia coli where as Proteus vulgaris was least predominant. Isolated bacteria were tested for sensitivity. Highest number of gram positive isolates were sensitive to Levofloxacin (81.21%) followed by Vancomycin (72.72%) and Ofloxacin (71.51%) whereas highest number (86.06%) of gram positive isolates shown resistance to Oxacillin (86.06%). Most of all the gram negative isolates were highly sensitive to Imipenem (90.76%) followed by Amikacin (73.84%) and Piperacillin/Tazobactam (68.46%), where as highest number (61.53%) of gram negative isolates shown resistance to ceftazidime. Amikacin is most effective drug against gram positive and gram negative bacteria.

Keywords: Wound infections, gram positive bacteria, gram negative bacteria, antibiotic sensitivity and bacterial resistance.

INTRODUCTION

Wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity caused by trauma, surgeries, burns, diabetic ulcers. Trauma may be accidental or intentionally induced. Wound provides a moist, warm and nutrient environment that is conductive to microbial colonization and proliferation that leads to serious bacterial wound infections and death.[1]

Wound can be infected by variety of bacterial pathogens; the common gram positive organisms are Staphylococcus

aureus and Staphylococcus epidermidis, the gram negative aerobic rods are Pseudomonas aeruginosa, the gram negative facultative anaerobes include Escherichia coli, Klebsiella species and Proteus species.

Infections of the surgical wound are one of the most common Hospital Acquired Infections [HAI] and are important cause of morbidity and mortality.

Burns provide a suitable site for bacterial multiplication. The burn is a richer and more persistent source of infection than the surgical wound because a large area of tissue is exposed for longer time. Staphylococcus aureus and Pseudomonas aeruginosa are the commonest isolates in most burns. The control of wound infections has become more challenging due to wide spread bacterial resistance to antibiotics and to greater incidence of infections caused by "Methicillin Resistant Staphylococcus aureus" [MRSA].[2] Infection in wound delays healing[3] and subsequent increased length of hospital stay also has economic consequences.[4]

Based on the literature given the present study was conducted to find the prevalence of etiological bacterial organisms for development of wound infection and also to identify the antibiotic susceptibility for therapeutic measures.

MATERIALS AND METHODS

The bacterial analysis of wound infection was carried out in the Department of Microbiology, GSL Medical College, Rajahmundry. The present study was done for a period of two years and one month that is from May 2011 to May 2013. During the period of study, 500 wound samples were collected from in and out patients of all the departments of GSL Medical College and General Hospital. The study was conducted with the approval of institutional ethical committee.

The collected samples were processed for direct microscopy and aerobic culture and sensitivity as per the standard protocol. Samples were inoculated on freshly prepared Nutrient Agar, Blood Agar and Mac Conkey Agar plates by streak plate technique and incubated at 35 + 2^{O}C aerobically for 16-18 hrs. Bacterial colonies are confirmed by cultural characters and biochemical reactions like gram staining, motility, oxidase test, coagulase test, indole test, methyl red, voges-proskauer test, citrate utilization test, urea hydrolysis test, Phenyl pyruvic acid test (PPA) and utilization of carbohydrates such as glucose, sucrose, lactose, maltose and mannitol. All the above media and reagents were procured from Himedia, Mumbai, India to conduct the study.[5]

Antimicrobial susceptibility of the isolates was performed on Mueller Hinton Agar plates by Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines.[5,6]

Antibiotics used for gram positive bacteria were Amoxycillin (10micrograms [mcg]), Amikacin (30mcg), Gentamycin (10mcg), Clindamycin (2mcg), Levofloxacin (5mcg), Netillin (30mcg), Oxacillin (1mcg), Ofloxacillin (5mcg) and vancomycin (30mcg). Antibiotics used for gram negative bacteria were Ampicillin (10mcg), Amikacin (30mcg), Aztreonam (30mcg), Ciprofloxacin (5mcg), Ceftazidime (30mcg), Gentamycin (10mcg), Gatifloxacin (5mcg), Imipenem (10mcg) and Piperacillin/Tazobactam (10mcg).

Six antibiotic discs per 90 mm Petri dish were placed on Mueller Hinton Agar with a gap of 25 mm from disc to disc by using all precautionary measurements and then the plates were incubated aerobically at 35 + 2^{O}C for 16-18 hrs to observe the zones of growth inhibition produced by the antibiotic and recorded immediately.[5]

All statistical analysis was performed by using "Statistical Package for the Social Sciences" (SPSS) software - 16 and MS Microsoft Excel 2007. The values were presented in percentages. Chi-square test was used for examining the association of categorical variables. For all statistical analysis $p < 0.05$ was considered statistically significant.

RESULTS

The results showed that the presence of gram positive bacteria like Staphylococcus aureus and Staphylococcus epidermidis, gram negative bacteria like Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris in wound infection. Staphylococcus aureus and Escherichia coli were most frequently isolated bacteria among gram positive and gram negative bacteria respectively.

Table 1 shows results of various authors regarding incidence of bacterial isolates from wound infections. Our present study showed that 425 bacterial isolates were isolated from 500 samples of wound samples.

Table 1. Various studies showing incidence of bacterial isolates.

Author	Samples	Bacterial Isolates
Sengupta et al [7]	103	71 (69 %)
Mahmood et al [8]	129	153 (119 %)
Shriyan et al [9]	100	84 (84 %)
Nwachukwu et al [10]	45	68 (151 %)
Dr. Sarvan Ricky R et al [11]	100	70 (70 %)
Sani R.A et al [12]	500	265 (53 %)
Present study	500	425 (85 %)

Table 2 shows number of various organisms isolated from different types wound infections. Staphylococcus aureus (102) was the most frequently isolated bacteria among all the isolates of wound infection.

Table 2 Different organism isolated from various types of wound infections.

Type of wounds	Samples screened [500]	Bacterial Isolates [425]	Staphylococcus aureus [102]	Escherichia coli [84]	Pseudomonas aeruginosa[80]	Klebsiella pneumoniae [66]	Staphylococcus epidermidis [63]	Proteus species [30]
Accidental Wounds	200	171	43	28	36	32	24	8
Burns	86	70	13	15	10	15	13	4
Diabetic Ulcers	214	184	48	39	34	19	26	18

Table 3 shows distribution of organisms among male and female patients. There was no statistically significant ($P>0.05$) association between organisms and gender.

Table 3. Distribution of organisms among male and female patients.

	Male	Female	Total	Chi-square	P Value
Gram Positive Organisms	103	62	165	1.24	0.265*
Gram Negative Organisms	176	84	260		
Total	279 (66%)	146 (34%)	425		

* P Value is statistically not significant (P>0.05)

Table 4 shows comparison between present and previous studies about isolates of wound infection. Most of the studies showed that Staphylococcus aureus was most predominant and Proteus vulgaris was least common bacteria in wound infections.

Table4. Comparison of isolated organisms between present and previous studies.

Author	S.aureus	E.coli	Pseudomonas aeruginosa	Klebsiella pneumoniae	S.epidermidis	Proteus vulgaris
Sengupta et al [7]	30	18	22	0	-	0
Mahmood et al [8]	65	19	21	16	-	8
Shriyan et al [9]	53	10	8	4	-	3
Nwachukwu et al [10]	33	10	25	0	-	10
Dr. Sarvan Ricky R et al [11]	7	4	45	10	-	4
Sani R.A et al [12]	-	39	64	42	-	49
Present study	102	84	80	66	63	30

S.aureus – Staphylococcus aureus, E.Coli – Escherichia coli and S.epidermidis - Staphylococcus epidermidis.

Table 5 shows that sensitivity of drugs were significantly (P<0.001) associated with gram negative organisms. Most of the gram negative isolates were highly sensitive to Imipenem (90.76%) followed by Amikacin (73.84%) and Piperacillin/Tazobactam (68.46%). Escherichia coli and Proteus vulgaris were showed highest resistance to ceftazidime, highest number of Pseudomonas aeruginosa were showed resistance to aztreonam where as highest number Klebsiella pneumoniae showed resistance to Ampicillin.

Table 5. Antibiotic Sensitivity and Resistance of Gram negative bacteria.

Antibiotic	E.Coli (84)		P.aeruginosa (80)		Kl.pneumoniae (66)		P.vulgaris (30)	
	S	R	S	R	S	R	S	R
Ampicillin	27 (32.14%)	57 (67.86%)	-	-	18 (27.27%)	48 (72.73%)	9 (30%)	21 (70%)
Amikacin	60 (71.42%)	24 (28.58%)	58 (72.5%)	22 (27.5%)	52 (78.78%)	14 (21.22%)	22 (73.33%)	8 (26.67%)
Aztreonam	-	-	32 (40%)	48 (60%)	-	-	-	-
Ciprofloxacin	28 (33.33%)	56 (66.67%)	45 (56.25%)	35 (43.75%)	21 (31.81%)	45 (68.19%)	21 (70%)	9 (30%)
Ceftazidime	25 (29.76%)	59 (70.24%)	42 (52.5%)	38 (47.5%)	24 (36.36%)	42 (63.64%)	9 (30%)	21 (70%)
Gentamycin	58 (69.04%)	26 (30.96%)	33 (41.25%)	47 (58.75%)	24 (36.36%)	42 (63.64%)	10 (33.33%)	20 (66.67%)
Gatifloxacin	47 (55.95%)	37 (44.05%)	-	-	28 (42.42%)	38(57.58%)	9 (30%)	21 (70%)
Imipenem	81 (96.42%)	3 (3.58%)	72 (90%)	8 (10%)	53 (80.30%)	13 (19.7%)	30 (100%)	0
Piperacillin/ Tazobactam	68 (80.95%)	16 (19.05%)	62 (77.5%)	18 (22.5%)	24 (36.36%)	42 (63.64%)	24 (80%)	6 (20%)
P Value			0.000*					

S - Sensitive; R – Resistance

* P value is highly significant (P<0.001)

E.Coli – Escherichia coli, P.aeruginosa – Pseudomonas aeruginosa, Kl.pneumoniae – Klebsiella pneumoniae, and P.vulgaris – Proteus vulgaris

Table 6 shows that drugs were significantly (P<0.001) associated with gram positive organisms. Highest number of gram positive isolates were sensitive to Levofloxacin (81.21%) followed by Vancomycin (72.72%) and Ofloxacin (71.51%) whereas resistant to Oxacillin (86.06%).

Table 6. Antibiotic Sensitivity and Resistance of gram positive bacteria.

Antibiotic	S.aureus (102)		S.epidermidis (63)	
	S	R	S	R
Amoxycillin	28 (27.45%)	74 (72.55%)	50 (79.36%)	13 (20.64%)
Amikacin	79 (77.45%)	23 (22.55%)	34 (53.96%)	29 (46.04%)
Gentamycin	80 (78.43%)	22 (21.57%)	36 (57.14%)	27 (42.86%)
Clindamycin	64 (62.74%)	38 (37.26%)	18 (28.57%)	45 (71.43%)
Netillin	68 (66.66%)	34 (33.34%)	26 (41.26%)	37 (58.74%)
Levofloxacin	82 (80.39%)	20 (19.61%)	52 (82.53%)	11 (17.47%)
Oxacillin	17 (16.66%)	85 (83.34%)	6 (9.52%)	57 (90.48%)
Ofloxacin	72 (70.58%)	30 (29.42%)	46 (73.01%)	17 (26.99%)
Vancomycin	82 (80.39%)	20 (19.61%)	38 (60.31%)	25 (39.69%)
P Value		0.000*		

S - Sensitive; R – Resistance
* P value is highly significant (P<0.001)
S.aureus – Staphylococcus aureus and S.epidermidis - Staphylococcus epidermidis

DISCUSSION

Staphylococcus aureus (102) was found to be highest incidence of all the isolates. This finding was similar with work done by Sengupta et al,[7] Mahmood et al,[8] Shriyan et al [9] Nwachukwu et al[10] and Kalakutakar et al. [13] But it was contrary with work done by, Dr. Sarvan Ricky R et al[11] which shows that Pseudomonas aeruginosa were showed highest incidence followed by Staphylococcus aureus.

Staphylococcus aureus showed the highest sensitivity to Levofloxacin (80.39%) and Vancomycin (80.39%) followed by Gentamycin (78.43%) whereas highest resistance shown against Oxacillin (83.34%). Shriyan et al[9] and Dr. Sarvan Ricky R et al[11] also reported as Staphylococcus aureus were most susceptible to Vancomycin (100%).

Escherichia coli (84) were found to be highest incidence among gram negative bacteria followed by Pseudomonas aeruginosa (80). This was similar with the findings of Siguan et al (1987),[14] Olayinka et al (2004) [15] and Sani R.A et al.[12] Proteus vulgaris (30) were showed least incidence of all the isolates from wound infection; this was similar with the findings of Dr. Sarvan Ricky R et al.[11]

Majority of gram negative isolates were sensitive to Imipenem (90.76%) followed by Amikacin (73.84%) and Piperacillin/Tazobactam (68.46%). Mahmood et al,[8] K Prabhat Ranjan et al[16] and Dr. Sarvan Ricky R et al[11] also reported that the gram negative isolates were found to be most susceptibility to Imipenem followed by Piperacillin/Tazobactam and Amikacin. Pseudomonas aeruginosa and Proteus species were also sensitive to Ciprofloxacin and this study was similar with that of reported by R.M Mordie et al.[17]

Escherichia coli (70.24%) and Proteus vulgaris (70%) showed resistance to Ceftazidime followed by Ampicillin. Pseudomonas aeruginosa showed resistance to Aztreonam (60%) followed by Gentamycin (58.75%).

Klebsiella pneumoniae showed resistance to Ampicillin (72.73%) followed by ciprofloxacin (68.19%), this was similar with that of reported by Anderl et al.[18] Highest numbers (62%) of gram negative isolates were shown resistance to ceftazidime.

The scenario of isolates and their antibiotic susceptibility pattern varies from place to place, time to time and patient to patient. It depends on the patients who were taking broad-spectrum of antibiotics as prophylaxis, infrequent usage of drugs, lower immune status, poor nourishment and age.

CONCLUSION

The findings of our study show that Staphylococcus aureus was found to be highest prevalence among all the isolates of wound infections. Majority of gram positive isolates were sensitive to Levofloxacin followed by Vancomycin and Ofloxacin, Gentamycin and Amikacin and resistance to Oxacillin. Most of gram negative isolates were highly sensitive to Imipenem followed by Amikacin and Piperacillin/Tazobactam where as resistance to ceftazidime. Amikacin is most effective drug against both gram positive and gram negative bacteria isolated from wound infections.

Development of resistance by certain bacterial strains in wound infections is due to improper antibacterial usage by patients which leads to sustained infection being common cause of morbidity, delay in recovery and subsequent increased length of stay in hospital. Patients may overcome

these problems by taking appropriate dosage of antibiotics and avoid irregular usage of drugs.

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