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Original Research Article

Multidrug Resistance Wound Pathogens- A Serious Challenge Ahead

Belgaum Vijayaraghavan Shamsundar

Asst. Professor of Microbiology, Department Of Microbiology, Mysore Medical College And Research Institute, Irwin Road, Mysore, Karnataka.

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ABSTRACT

Objective: Increasing number of reports had documented the continued emergence of drug resistance among gram positive and gram negative clinical isolates to common antimicrobial drugs, world-wide. This study investigated the antimicrobial resistance patterns of gram positive and gram negative culture isolates obtained from hospitalized patients.
Methods: Between Feb. 2013 and July 2013, a total of three hundred and twenty three organisms belonging to different genus were isolated from pus and other exudates samples and fully characterized by standard bacteriological procedures. Antimicrobial susceptibility patterns of each isolate were carried out by the Kirby- Bauer disk diffusion method as per guidelines of CLSI.
Results: Majority of isolates were Staphylococcus aureus (120, 83.75%). The other organisms isolated were E. coli, pseudomonas spp., Klebsiella, acinetobacter, proteus, enterobacter, citrobacter, coagulase negative staphylococcus (CONS), streptococci, enterococci, and other Non fermenters including Acinetobacter spp. The isolated pathogens showed resistance to amikacin (17.25%), ciprofloxacin (27.59%) and cefoperazone -sulbactum (34.48%). Resistance rates to Co-trimoxazole, piperacillin, ceftriaxone and chloramphenicol varied from 51.00% to 73.00%. All the isolates were susceptible to imipenem. 30 (20.69%) of P. aeruginosa isolates were multi-drug resistant.
Conclusion: This study reveals that a variety of bacterial pathogens are responsible for wound infection Staphylococcus aureus was found to be the most common organism isolated. Majority of the bacterial isolates were resistant to almost all the antimicrobials employed. Among all the bacterial isolates, Escherichia coli were found to be highly resistant to commonly used antibiotics. High rate of multiple antibiotic resistance among both gram positive and gram negative bacterial species have been observed, which may have impact on prolonging wound healing and increasing the cost of therapy to patients. Thus, it is highly recommended to perform antimicrobial susceptibility testing before administration of antibiotics. In our study P. aeruginosa showed reduced sensitivity to commonly used antibiotics like ampicillin, doxycycline, nalidixic acid, and tetracycline, except ciprofloxacin, norfloxacin (100%), and gentamicin (82%). Ciprofloxacin and norfloxacin has been stated to be the most potent oral drug available for the treatment of P. aeruginosa infections.

Keywords: antimicrobial resistance, clinical isolates, tertiary care hospital

INTRODUCTION

Infectious diseases have been an important cause of morbidity and mortality throughout our history. With the expansion of the antibiotic era during the 20th century, there was a growing confidence that the need for infectious disease specialists would all but disappear. However, no one could

have predicted the impact that an increasing immunocompromised population would have on the resurgence of infectious diseases during the last three decades. Furthermore, the ability of bacterial pathogens to adapt and to overcome the challenges of antibiotics in their environment has been nothing short of impressive. We are now faced with a growing population of pan-resistant bacteria that threaten to move us into what some consider the "postantibiotic era" of infectious diseases.

Some of the more problematic drug-resistant pathogens encountered today include methicillin-resistant Staphylococcus aureus, multidrug-resistant Streptococcus pneumoniae, and vancomycin-resistant Enterococcus spp. among the gram-positive bacteria and multidrug-resistant Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa among the gram-negative bacteria.. Unfortunately, selection of the most appropriate antibiotic is complicated by the ability this pathogenic microorganisms and especially Pseudomonas strains develop resistance to multiple classes of antibacterial agents, even during the course of treating an infection.

Despite technological advances in surgical wound infection still has been regarded as the most common nosocomial infection, especially in patients undergoing surgery. [1] An important cause of illness wound infection results in prolonged hospital stay and increased trauma care and treatment costs; in general, wound management practices become more resource demanding. [2] The severity of complications depends largely on the infecting pathogen and site of infection. Wound infections have been a problem in the field of medicine for a long time. Advances in control of infections have not completely eradicated this problem because of development of drug resistance. [3] 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. [4] Antimicrobial resistance can increase complications and costs associated with procedures and treatment. [5] Knowledge of the causative agents of wound infection has proven to be helpful in the selection of appropriate antimicrobial therapy and on infection control measures taken in health institutions. [6] Hence, the present study was designed to update knowledge on bacteriology of wounds and the sensitivity pattern of the causative agents to the common antibiotics used in Medical College Teaching Hospital, In this article we have taken a study on drug susceptibility pattern of culture isolates from wound specimen samples received in the laboratory .as this microbes pose serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome

MATERIALS AND METHODS

The study was conducted over a period of six months (Feb 2013to july2013) at the tertiary care hospital in south India. Samples were obtained from patients who were hospitalized and from the patients attending out patients department. The specimens obtained were from exudates samples from any lesion which was present (e.g. Burn wound, non-healing ulcer, post-operative wounds). A total of 415 samples were obtained from different sources. These specimens were inoculated onto the primary isolation media like blood agar oxidase nitrate and catalase tests and other biochemical tests were done to identify enterobacteriaceae family, pseudomonas and other Non fermenters, and for the identification of gram positive isolates. The Kirby Bauer Method using the disc diffusion

technique was the procedure of choice for antibiotic sensitivity testing.

Suspensions of the isolates of 0.5 McFarland turbidity standard were made and Mueller Hinton Agar (MHA) plates were inoculated. Antibiotic discs of Imipenem (10µg), Amikacin (30 µg), Gentamicin (10µg), Piperacillin-tazobactam (110µg), Aztreonam (30µg), Cefoperazone (75µg), Ceftazidime (30 µg), Ciprofloxacin (5 µg), were applied on the plates. The plates were then incubated at37°Cfor16to18 hours and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Clinical and Laboratory Standard Institute (CLSI).*CLSI*; 2009. [7] Gram positive bacteria were tested against selected 11 antibiotics.

Data were edited, cleaned, entered and analyzed using statistical package for social science (SPSS) version 20. Descriptive analyses such as frequencies and cross tabulations were used. The chi-square test was employed to compare the association of socio-demographic data, location with wound infection status of the patients. P-value of less than 0.05 was considered to indicate statistically significant differences. The result was presented using tables and charts.

RESULTS

Table 1 Wound infection and socio-demographic characteristics of the patients

Demographic characters	Infected No. (%)	Not infected No. (%)	Total No. (%)	p
Sex				
Male	170 (56.63)	51(55.43)	221 (55.25)	0.3
Female	153 (47.36)	41 (44.57)	194 (46.75)	
Total	**323 (100)**	**92 (100)**	**415 (100)**	
Age in years				
≤ 20	48 71.64	19(28.36)	67(16.14)	
21-40	122 (73.94)	43 (26.06)	165 (39.76)	
41-60	102 (83.60)	20(16.39)	122(29.40)	
				0.09
≥60	51(83.61)	10(16.39)	61 (14.70)	
Total	**323 (87.3)**	**92(12.7)**	**415 (100)**	

A total of 415 specimens were collected from patients with clinical evidence of wound infection (patients with complaints of discharge, pain, swelling, foul smelling and chronic wound) from Feb 2013 to July 2013. The subjects included 221 (55.25%) males and194 (46.75%) females. (Table-1)

Table-2 Number of organisms isolated from various types of wounds.

Type of wound	number	percentage
Trauma	120	(37.15)
Postoperative wound	75	(23.21)
Abscess	65	(20.12)
Ulcers	20	(06.19)
Burn wound	30	(09.28)
Diabetic foot ulcers	13	(04.02)
Total	323	(100.0)

Bacterial profile

Of the 415 swabs 323 (77.83%) were culture positive for bacterial pathogens, while 78 (12.6%) were bacteriologically sterile. The presence of only one species isolated from each sample was222 (68.73%) while, more than one species were isolated from (31.27%) of the total swabs. A total of 434 bacterial isolates were obtained out of which, 271(62.44%) were gram negative while163 (37.56%) were gram positive organisms. S. aureus was the predominant organism isolated 124 (28.57%), followed by Escherichia coli (E. coli) 81 (18.66%), P. aeruginosa 53(12.21%) Klebsiella spp. 44(10.14%) Acinitobacter spp. 37 (8.52), Proteus spp. 31 (7.14%), Enterococci spp. 15(3.46%) Enterobacter spp. 14(3.22%) (Coagulase negative Staphylococci 12 (2.76%), streptococci spp. 12 (2.76%) and Citrobacter spp. 11 (2.53%), (table- 3).

Table-3 Various organisms isolated and their percentage

Name of the organism	Total number	percentage
s.aureus	124	28.57%,
E.coli	81	18.66%,
Pseudomonas	53	12.21%
Klebsiella	44	10.14%
Acinobacter spp.	37	8.52
Proteus	31	7.14%
enterococci	15	3.46%
Enterobacter spp.	**14**	3.22%
streptococci spp.	**11**	2.76%
Citrobacter spp.	12	2.53%
TOTAL	**434**	**100%**

Table 4. Antibiotic resistance pattern of gram positive wound isolates.

Name of the organism	amx	AT	CO	CF	CI	CE	CX	CD	G	LZ	VA
s.aureus n=124	114 92%	69 55.64%	31 25%	51 41.12%	110 88.70%	115 92.74%	50 40.32%	35 28.32%	42 33.02%	08 6.45%	08 6.45%
Cons n=12	11 91.66%	06 50%	05 41.66%	08 66.66%	10 83.33%	10 83.33%	09 75%	06 50%	03 25%	04 33.33%	05 41.66%
enterococci n=15	09 60%	14 93.33%	ND	13 86.66%	13 86.66%	13 86.66%	12 80%	14 93.33%	14 93.33%	04 26.66%	04 26.66%
Streptococci n=12	07 58.33%	11 91.66%	ND	09 75%	10 83.33%	08 66.66%	12 100%	11 91.66%	04 33.33%	00	00

Abrevation-amx=amoxacillin; AT=azithromycin; co=cotrimaxazole; CF=ciprofloxacin; CI=ceftriaxone; CE=cefatoxime; CX=cefoxitine; CD=clindamycin; G=gentamycin; LZ=linozolide; VA= vancomycin

Table-5. Antibiotic resistance pattern of the Gram negative wound isolates.

Antibiotic discs	E.coli	Pseudomonas aeruginosa	Klebsiella	Acinobacter spp.	Proteus	enterobacter spp.	Citrobacter spp.
amoxacillin;	66 81.40%	ND	30 68.18%	30 100%	21 67.74%	14 100%	06 54.54%
Amikacin	24 29.63%	14 26.41%	10 22.72%	06 16.21%	06 19.35%	02 14.28%	01 9.09%
ciprofloxacin;	54 66.66%	24 45.28%	30 68.18%	30 81.08%	09 29.03%	01 7.14%	01 9.09%
ceftazidime	60 74.04%	24 45.28%	28 63.63%	24 64.86%	20 64.86%	04 28.5%	05 45.45%
cefatoxime	65 80.25%	40 75.47%	30 68.18%	24 64.86%	21 67.74%	05 35.71%	06 54.54%
gentamycin	26 32.09%	28 52.83%	27 61.36%	18 48.64%	21 67.74%	01 7.14%	02 18.18%
Imipenem	08 9.87%	05 9.43%	08 18.18%	03 8.10%	01 3.22%	01 7.14%	01 9.09%
Piperacillin-Tazobactam	14 17.28%	10 18.86%	26 59.09%	18 48.64%	05 16.12%	01 7.14%	01 9.09%
cotrimaxazole	42 51.85%	38 71.69%	35 79.54%	ND	13 41.93%	07 50%	06 54.54%

DISCUSSION

In the present study 77.83% of pus culture was found to be positive for bacterial growth. The incidence of wound infection was more common in males 170 (56.63) than in females 153 (47.36) which is in accordance with various studies done in different region. [5,8,9] This might be explained by the fact that traditionally, in this country mainly males are involved in occupations such as farming, construction works, transportation and industry works where the likely exposure to trauma is Common.

In our study, S. aureus 124 (28.57%), and E. coli Escherichia coli (18.66%), were the predominant organisms isolated from wound infections. A number of reports done previously on wound infection from different parts of the world indicated that S. aureus and E. coli were the most frequent isolates. [10-13] The high prevalence of S.

aureus infection may be because it is an endogenous source of infection. Infection with this organism may also be due To contamination from the environment e.g. contamination of surgical instruments. With the disruption of natural skin barrier S. aureus, which is a common bacterium on surfaces, easily find their way into wounds. Coagulase Negative Staphylococci (CONS) accounted for 14.5% of the organisms isolated from wounds in this study. This is not unexpected since the organism is a commensal or normal flora on the skin. Several investigations have reported these organisms as common contaminants of wounds, [12,14]

Resistance to the selected antimicrobials was very high. The average resistance of the isolates to all the antibiotics in gram positive cocci was (99%) and gram negative bacilli (100%). This is similar to the study done in Ethiopia with average resistance of gram positive cocci isolates (100%) and gram negative bacilli isolates (95.5%) respectively. [12] The overall multiple drug resistance (two and above antimicrobial classes) of the isolates in this study was 85%.

Which was in line with previous study done in different parts of the world? [11,15,16] High resistance of the isolates to antibiotics may be due to practicing self medication, lack of diagnostic laboratory services or unavailability of guideline regarding the selection of drugs thereby which lead to inappropriate use of antibiotics. In the determination of the susceptibility of S. aureus on eleven selected antibiotics by disk diffusion technique showed that S. aureus tend to be resistant to a wider spectrum of antibiotics. In this studies S. aureus was highly resistance to. Amoxicillin. (92%). This was consistent with study done by various workers. [8,17-19]

The sensitivity to other group of drugs is as fallows. vancomycin (93.55%), ciprofloxacin (58.88%), and gentamicin (66.94%). This finding is in agreement with the work done by Shriyan A et al., and other workers [19-24] who reported that clinical Staphylococci are 100% sensitive to vancomycin. coagulase negative Staphylococci were 59% sensitive to vancomycin, sulphamethoxazole - trimethoprim (59%), gentamicin (75%) and ciprofloxacin (34%). This finding was comparable with the previous studies done in different parts of the world. [8,25] The same organism was remarkably resistance to ampicillin (92%), cefataxime 83.33% ciftriaxone, (83.33%) and clindamycin (50%). This finding was comparable with study done in other parts of the world. [8,17,18,26] high percentage of susceptibility of gram positive bacteria to vancomycin and linozolide and aminoglycosides (gentamicin) may be due to lesser use of these antibiotics as a result of their less availability, cost and toxic effect respectively

Escherichia coli (81) were found to be highest among gram negative bacteria followed by Pseudomonas aeruginosa (53). This was similar with the findings of Siguan et al. [27] Olayinka et al (2004) [28] and Sani R.A et al. [29]

Majority of gram negative isolates were sensitive to Imipenem followed by Amikacin and Piperacillin/Tazobactam. Mahmood et al, [30] K Prabhat Ranjan et al [31] and Dr. Sarvan Ricky R et al [32] 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 showed sensitivity of 54.72% and 70.97% to Ciprofloxacin and this study was similar with that of reported by R.M Mordie et al. [33]

In this study, the E. coli isolates were highly resistant to ampicillin (81.40%). This finding was comparable with the previous studies done by Mohammedaman Mama et al. [34] High percentage of Escherichia coli

(74.07%) and Proteus spp. (64.51%) showed resistance to Ceftazidime. Pseudomonas species showed highest resistance to cefatoxime fallowed by Gentamycin. (75.47% and 52.83% respectively)

Klebsiella spp. showed resistance to Ampicillin (68.18%) followed by ciprofloxacin (68.18%), this was similar with that of reported by Anderl et al. [35] Highest numbers (62%) of gram negative isolates were shown resistance to ceftazidime.

High percentage of enterobacter spp. showed resistance to ampicillin, cotrimaxaxole, and ceftazidime. (100%, 50%, and 28.5% respectively). Enterobacter showed high sensitivity to ciproflaxcin (93%), gentamycin(93%) and Piperacillin-Tazobactam(93%) this findings are similar to study done by Mulugeta K. Azene et al. [36]

Citrobacter spp. showed highest resistance again to penicillin group that is ampicillin (54.54%), followed by ceftazidime (45.45%), cefataxime (54.54%) and cotrimaxazole (54.54%) whereas ciprofloxacine, amikacine and Piperacillin-Tazobactam were highly sensitive (all the drugs showed 91% sensitivity). These findings are again similar to study done by Mulugeta K. Azene et al. [36]

Acinobacter spp. (non fermenter group) were highly resistant to ampicillin (100%), ciprofloxacin (81%), Ceftazidime and cefataxime (both64.86%), gentamycin (48.64%), and Piperacillin –Tazobactam (48.65%). Whereas Acinobacter spp. showed high sensitivity to imepenam (93%) and amikacine (84%).this findings are similar to the figures mentioned in the review literature written by CareKerry Montefour et al. [37]

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 .The relatively high resistance of gram positive and gram negative organisms isolates to commonly used antibiotics as recorded in this study gives course for worry, especially in the developing nations were most of these antibiotics still serve as first line drugs. The inordinate accessibility of antibiotics in shops and open markets as well as consumption of drugs without proper medical prescription- a common practice in resource poor countries, is probably an important factor worthy of consideration, if any success in the fight against microbial resistance to drugs is anticipated routine sensitivity screening of antibiotics before prescription is suggested. Also, the urgent need for health systems in the developing nations to strategize on appropriate drug administration channel based on their peculiar circumstances is a compelling necessity.

CONCLUSION

This study reveals that a variety of bacterial pathogens are responsible for wound infections *Staphylococcus aureus* was found to be the most common organism isolated. Majority of the bacterial isolates were resistant to almost all the antimicrobials employed. Among all the bacterial isolates, *Escherichia coli* was found to be highly resistant to commonly used antibiotics

High rate of multiple antibiotic resistance among both gram positive and gram negative bacterial species have been observed, which may have impact on prolonging wound healing and increasing the cost of therapy to patients. Thus, it is highly recommended to perform antimicrobial susceptibility testing before administration of antibiotics.

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