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Study of Bacteriological Etiology of Wound Infections.

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ABSTRACT

Infection is one of the major barriers for the process of healing of the wound. Hence it has an adverse impact on the patient's quality of life. Any wound is at risk of becoming infected and most frequently wound colonisation is poly microbial. This study was done to know the common causative agents of wound infection and their varying antibiotic susceptibility patterns. A total of 100 samples were included in the study. They were subjected to routine manual culture methods, biochemical investigations and antibiogram. Various pathogenic microorganisms were isolated from the wound infections. Out of which *Staphylococcus aureus* (31%) was the commonest, followed by *Pseudomonas aeruginosa* (20%), Coagulase Negative *Staphylococcus* (CONS) (18%), *Proteus* sp (12%), *Escherichia coli* (10%), *Klebsiella* sp (9%). Amikacin was found to be the susceptible drug among most of the gram negative isolates. The overall multi drug resistance pattern of gram negative isolates was observed to be 53.3%. Extended-spectrum Beta-Lactamase producing bacteria are being increasingly reported. Hence infection control measures are of great importance in the current era of highly resistant microbial population.

Keywords: wound infections, etiology, antibiogram, muti drug resistance

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INTRODUCTION

An important cause of mortality and morbidity in hospitalised patients is infection [1]. The primary and major function of an intact skin is to protect the underlying tissue from colonization of potential pathogens that are present freely in the environment and skin surface [2]. Therefore following a wound, there is exposure of subcutaneous tissue leading to development of a nutritious, moist, warm and favourable environment for the colonization and multiplication of microorganisms. Any wound is at risk of becoming infected. But the major risk factors for wound infection includes old age, prolonged hospital stay, immunocompromised state, indwelling devices like intravenous catheters, urinary catheters, endotracheal intubation, etc., irrational use of antibiotics and other preexisting conditions like malignancies, renal failure[3].

Wound infections are most often poly microbial [4]. The pathogenic microorganisms include both gram positive or gram negative bacteria. The causative microorganisms vary with the site of infection. A good healthy environment is required for the occurrence of a normal physiological process of healing. This can be achieved by sterilizing the damaged tissue and making it free from microbial colonization [6]. This has led to the continued use of antibiotics, which in turn has caused a selective pressure and finally the emergence of drug resistance. But however there is a constant shift in the pattern of drug resistance in the microorganisms. Hence it is necessary to have knowledge about the etiologic agents and their antimicrobial susceptibility in a particular region or even a locality which will be useful for the selection of empirical antimicrobial therapy.

MATERIALS AND METHODS

This study was done in a tertiary care hospital in Chennai from December 2013 to April 2014. A total of 100 wound samples were taken in the study. The wound swabs and pus samples collected from the wounds were cultured in Nutrient agar, MacConkey agar and blood agar plates as surface streaks. The colonies grown after an overnight incubation were picked up and subjected to Gram staining, hanging drop, coagulase, catalase and oxidase tests. Further they are subjected to biochemical reactions.

Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics used for the gram negative isolates are Amikacin (30µg), Gentamycin (30µg), Aztreonam (30µg), Ceftazidime (30µg), Nitrofurantoin (300µg), Cefuroxime (30µ), Nalidixic acid (30µg), Cefixime (5µg), Cefdinir (5µ), Cefotaxim (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg). A lawn culture of the isolates was made on Muller Hinton agar plate and antimicrobial susceptibility testing is performed. The sizes of the zone of inhibition were measured and susceptibility is interpreted according to the recommended CLSI guidelines.

In our study, Multi Drug Resistance (MDR) was determined according to the criteria set by CLSI guidelines against antimicrobials by disc diffusion method. Penicillin class (ex. Ampicillin), Cephalosporin class (ex. Ceftriaxone, cefixime), Aminoglycosides (ex. Gentamycin), Tetracycline class (ex. Tetracycline), Fluoroquinolones class (ex. Ciprofloxacin), Quinolones class (ex. Nalidixic acid), Folate pathway inhibitors (ex. Cotrimoxazole) and Phenicols class (ex. Chloramphenicol). Resistance against 2 or more antibiotic classes is considered as multi drug resistance[7].

RESULTS

100 samples were included in our study. The incidence of different pathogenic microorganisms isolated from the various samples is described in Table 1.

Table 1: Incidence and distribution of the pathogens

ISOLATES	PERCENTAGE (%)
Staphylococcus aureus	31
Pseudomonas aeruginosa	20
Coagulase Negative Staphylococcus	18
Proteus species	12
Escherichia coli	10
Klebsiella species	9

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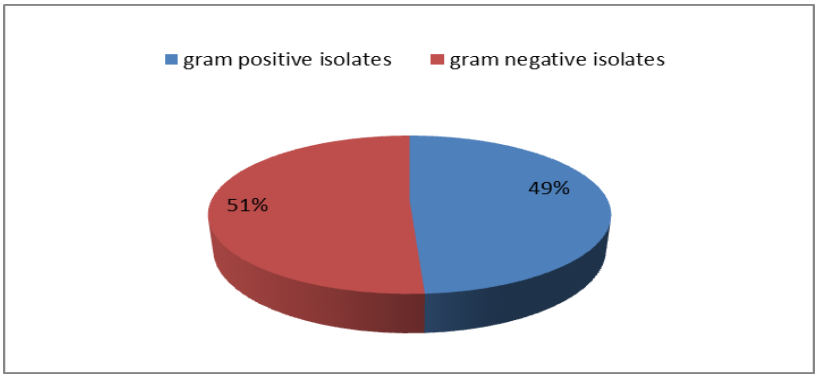

Figure 1: Order of prevalence of gram positive and gram negative isolates

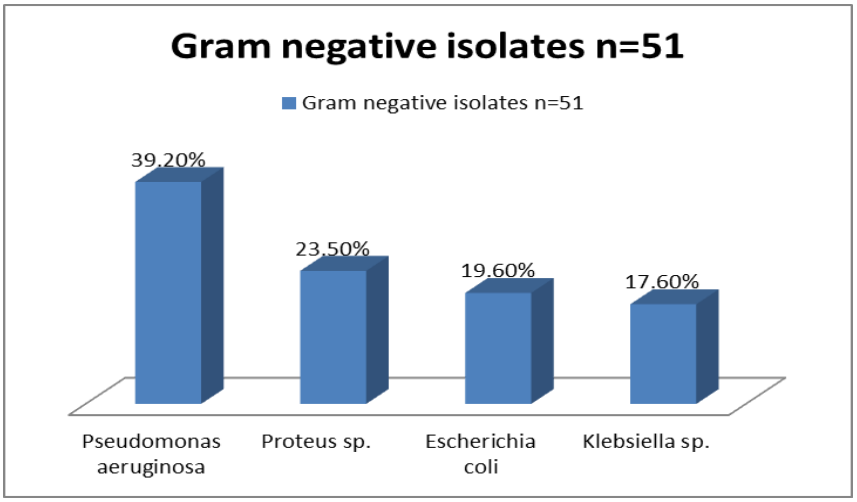

Figure 2: Order of prevalence of various gram negative isolates

Out of 20 Pseudomonas isolates, 13 were resistant to atleast 3 antibiotics tested. Therefore 65% of Pseudomonas species were considered to be multi drug resistant. Similarly out of 12 Proteus isolates, 5 were resistant to a minimum of 3 antibiotics tested. Therefore 41.6% of Proteus species were considered to be multi drug resistant. ESBL production was found out in 70% of Escherichia coli isolates and 66.6% of Klebsiella species.

Table 2: Percentage of antimicrobial susceptibility pattern of gram negative isolates

Antibiotics	Pseudomonas sp. (n=20)		Proteus sp. (n=12)		E.coli (n=10)		Klebsiella sp. (n=9)	
	R	S	R	S	R	S	R	S
Ceftriaxone	75%	25%	58.3%	41.6%	70%	30%	70%%	30%
Cefotaxime	65%	35%	100%	0	69%	31%	71%%	29%
Cefdinir	55%	45%	50%	50%	100%	0	100%	0
Cefixime	90%	10%	41.6%	58.3%	100%	0	100%	0
Cefuroxime	70%	30%	66.6%	33.3%	100%	0	100%	0
Ciprofloxacin	50%	50%	33.3%	66.6%	60%	40%	22.2%	77.7%
Gentamycin	20%	80%	33.3%	66.6%	70%	30%	66.6%	33.3%
Amikacin	10%	90%	25%	75%	10%	90%	11.1%	88.8%
Aztreonam	45%	55%	75%	25%	100%	0	100%	0
Nitrofurantoin	100%	0	16.2%	83.3%	30%	60%	33.3%	66.6%
Nalidixic acid	50%	50%	100%	0	10%	0	100%	0
Ofloxacin	55%	45%	50%	50%	50%	50%	33.3%	66.6%
Norfloxacin	70%	30%	58.3%	41.6%	70%	30%	44.4%	55.5%
Ceftazidime	65%	35%	41.6%	58.3%	70%	30%	33.3%	66.6%

DISCUSSION

The most prevalent microbial population responsible for wound infections are reported to be *Staphylococcus aureus* and *Pseudomonas aeruginosa* [5]. In our study also, *Staphylococcus aureus* (31%) and *Pseudomonas* sp., (20%) were the commonest microorganisms isolated from wound infections. This is in accordance with a number of previous studies conducted in various parts of the world. This is followed by Coagulase Negative Staphylococcus, which accounted for about 18% of the organisms isolated. It seems to be usual because the organism occurs as a normal commensal of skin. Various investigations have reported that they are normal skin contaminants [18,19]. *Proteus* (12%), *E.*coli (10%), *Klebsiella* species (9%) isolates were the other pathogens reported in our study. These reports were similar to the findings of Mahmood *et al* [16]

Multi drug resistance have been exhibited more in number by the isolated organisms. The overall multiple drug resistance in our study was 60.8%. This is comparable with the work of Andargachew *et* al., who reported the multiple drug resistance rate of about 58.5% in their study [21]. Increasing rate of resistance to pathogens was due to self-medication practice, unavailability of proper guidelines for the selection of drugs for empirical use and lack of diagnostic laboratories. Emergence of ESBLs is due to increasing use of cephalosporins in daily clinical practices.

Most of the *Pseudomonas* sp. is sensitive to Amikacin (90%) and Gentamycin (80%) when compared with Van Eldere *et al*, 2003. Nitrofurantoin is resistant to all 20 *Pseudomonas* isolated. Hence on the whole, 65% of *Pseudomonas* sp., are found to be multi drug resistant,

Among the 12 Proteus species isolated, most of them were sensitive to Nitrofurantoin (83.3%), Amikacin (75%), Gentamycin (66.6%) and Ciprofloxacin (66.6%). This correlates with the study done by C. Manikandan *et* al. 100% resistance were identified in Cefotaxime and Nalidixic acid.

Escherichia coli showed 100% resistance to Cefdinir, Cefuroxime, Cefixime, Aztreonam. 90% sensitivity to Amikacin and 60% sensitivity to Nitrofurantoin was observed. Resistance to Ceftriaxone and Ceftazidime was observed in about 70% of the isolates indicating ESBL production in Escherichia coli[8]. Resistance to Ceftazidime and susceptibility to the combination of Ceftazidime and Clavulanic acid confirms ESBL production. Another sensitive drug in ESBL production is Cefpodoxime[9]. Resistance to Cefotaxime also indicates ESBL according to NCCLS guidelines 2000[10].

Klebsiella sp., showed 100% resistance to Cefdinir, Cefixime, Cefuroxime, Aztreonam, Nalidixic acid. 88.8% sensitivity is observed in Amikacin, 77.7% sensitivity to Ciprofloxacin and 66.6% susceptibility to Nitrofurantoin and Ofloxacin. ESBL production is observed in 66.6% of the isolates. But these findings were in contrast with the previous study done by C. Manikandan *et* al, 2013. Prevalence of ESBL producing Klebsiella sp. is reported more frequently now a days[11-14]. Resistance to Ceftazidime is found to be a good indicator of ESBL production in Klebsiella [15].

CONCLUSION

The most common microorganism isolated from wound infections in our study is Staphylococcus aureus followed by Pseudomonas and other gram negative bacteria. In the present study, we have observed high level of resistance exhibited by the isolates to various antibiotics. Rate of isolation of multi drug resistant pathogens and ESBL producing bacteria is increased. Regular antimicrobial susceptibility surveillance is necessary to help the physicians in selecting appropriate empirical antibiotic therapy. Therefore the mortality and morbidity occurring due to wound infections can be minimised.

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