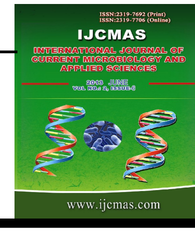

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Original Research Article

Antibiotic susceptibility of bacterial strains isolated from wound infection patients in Pattukkottai, Tamilnadu, India

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A B S T R A C T

Keywords

Wound infections; Bacteriology; Multi-drug resistant; susceptibility patterns.

The distribution of various pathogens causing wound infection was evaluated in Pattukkottai, Thanjavur district, Tamilnadu. A total of seventy wound swab specimens were collected and cultured, of which all samples showed bacterial growth. Six different species of bacteria were isolated. *Pseudomonas aeruginosa* (42.9%) and *Staphylococcus aureus* (24.3%) were the most common organisms followed by *Staphylococcus epidermidis* (15.7%), *Proteus* spp. (8.6%), *E.coli* (5.7%) and *Klebsiella pneumoniae* (2.8%). The antibiotic susceptibility test of the bacterial isolate was performed by Kirby-Bauer disk diffusion method. Majority of the bacterial isolates were resistant to almost all the antimicrobials employed. Among all the bacterial isolates, *Pseudomonas aeruginosa, E.coli* and *Klebsiella pneumoniae* were found to be highly resistant to commonly used antibiotics. High rate of multiple antibiotic resistances was observed in both Gram positive and Gram negative bacterial species recovered.

Introduction

Wound infections are one of the most common hospital acquired infections and are an important cause of morbidity and account for 70-80% mortality (Gottrup *et al.*, 2005; Wilson *et al.*, 2004). Wound infections can be caused by different groups of microorganisms like bacteria, fungi and protozoa. However, different microorganisms can exist in polymicrobial communities especially in the margins of wounds and in chronic wounds (Percevil and Bowler, 2001). 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* ((Rajendra Gautam *et al.*, 2013; Tayfour *et al.*, 2005).

Wound infections have been a problem is the field of medicine for a long time. The presence of foreign materials increases the risk of serious infection even with relatively small bacterial inoculums (Rubin, 2006). Advances in control of infections have not completely eradicated this problem because of development of drug resistance (Thomas, 1981). 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 (Elmer *et al.,* 1997; Sani *et al.,* 2012; Mulugeta and Bayeh, 2011). Antimicrobial resistance can increase complications and costs associated with procedures and treatment (Anguzu and Olila, 2007).

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 in Pattukkottai area, Tamil Nadu as well as update the clinicians in the various antimicrobial alternatives available in the treatment of wound infections.

Materials and Methods

Specimen collection

Samples were collected from the seventy patients with complaints of discharge, pain, swelling, foul smelling, delayed and non healing wound infection. The wound samples were collected by using a sterile cotton swab, the inner surface of the infected area was swabbed gently and then the swabs were transported to the laboratory.

Bacteriology

In the laboratory, each sample was inoculated on McConkey agar, Nutrient agar and Blood agar. The inoculum on the plate was streaked out for discrete colonies with a sterile wire loop. The culture plates were incubated at 37°C for 24 hours and observed for growth through the formation of colonies. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Sharma (2008).

Antibiotic susceptibility testing

Disc diffusion testing was performed according Kirby-Bauer method, as described in the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 2000, 2002), using discs (Himedia) containing 30 μg Amikacin, 10 μg Amoxicillin, 10 μg Ampicillin, 15 μg Azithromycin, 30 μg Cefazolin, 30 μg Cefaclor, 30 μg Cefotaxime, 30 μg Ceftazidime, 30 μg Ceftriaxone, 30 μg Cefuroxime, 05 μg Ciprofloxacin, 30 μg Doxycycline, 15 μg Erythromycin 10 μg Gentamycin, 05 μg Ofloxacin and 30 μg Tobramycin. A lawn of test pathogen (1ml of an 18 hours peptone broth culture) was prepared by evenly spreading 100μl inoculums with the help of a sterilized spreader onto the entire surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. Then, some commercially available antibiotic discs were gently and firmly placed on the agar plates, which were then left at room temperature for 1 h to allow diffusion of the antibiotics into the agar medium. The plates were then incubated at 37°C for 24 hours. If an antimicrobial activity was present on the plates, it was indicated by an inhibition zone. The diameter of the inhibition zones was measured in millimeter at 24 hours using a scale. An organism was interpreted as highly susceptible if the diameter of inhibition zone was more than 19 mm, intermediate if diameter was 15-18 mm and resistant if the diameter was less than 13 mm. The intermediate readings were considered as sensitive in the assessment of the data.

Result and Discussion

Samples were collected from the seventy patients with complaints of discharge, pain, swelling, foul smelling and non healing wound infection. Majority of the patients with ulcer wounds were diabetic.

The various types of bacteria isolated from wound culture were shown in table 1. The results showed that *Pseudomonas aeruginosa* was the predominant (30 isolates; 42.9%) followed by *Staphylococcus aureus* (17 isolates; 24.3%), *Staphylococcus epidermidis* (11 isolates; 15.7%). *Proteus spp.*, (6 isolates; 8.6%), *E. coli* (4 isolates; 5.7%) and *Klebsiella* spp. (2 isolates; 2.8%).

Antibiotic sensitivity of the isolated organisms was determined by standard disk diffusion method (Table.2). As indicated in table-2, *P. aeruginosa* exhibited high resistance to doxycycline (100%), ampicillin, amoxicillin (90%), erythromycin, cefaclor (80%), cefuroxime, cefazolin (73.3%), ceftazidime, azithromycin (26.7%). *P. aeruginosa* showed the highest antibiotic resistance rate and was significantly resistant to most of the antibiotics. However the third generation antibiotics cefotaxime, amikacin, gentamycin, ciprofloxacin, tobramycin and ofloxacin proved to be very effective against *P. aeruginosa.*

Table.1 The various species of bacteria isolated from wound culture.

Organisms	No of strains	Frequency %
P. aeruginosa	30	42.9
S. aureus	17	24.3
S. epidermidis	11	15.7
Proteus spp.	6	8.6
E. coli	4	5.7
Klebsiella spp.	2	2.8
Total	70	100

Staphylococcus aureus isolates exhibited highest susceptibility against amikacin, tobramycin, ceftazidime, ceftriaxone and cefotaxime 100% respectively. Other agents were also effective except ampicillin, amoxicilin and azithromycin. Against these agents wounds isolates indicated a resistance pattern of 85.7%, 84.3% and 50% respectively. The *Staphylococcus epidermidis* isolates showed very high sensitivity to amikacin, ofloxacin, ciprofloxacin, tobramycin, ceftazidime, ceftriaxone and cefotaxime proved to the most effective antibiotic exhibiting 100% sensitivity for wound isolates.

Escherichia coli showed 100% resistance to ampicillin, amoxicillin, cefaclor, doxycyline and 87.5% resistant to

Table.2 Antibiotic sensitivity/resistance pattern (%) of the wound isolates.

Antibiotics	Result	Bacterial isolates					
		Pseudomonas aeruginosa	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Proteus* spp.	*E. coli*	*Klebsiella* spp.
Amikacin	R	0	0	0	0	0	0
	S	100	100	100	100	100	100
Amoxicillin	R	90	50	76.7	100	100	100
	S	10	50	23.3	0	0	0
Ampicilin	R	90	85.7	76.7	100	100	100
	S	10	14,3	23.3	0	0	0
Azithromycin	R	26.7	84.3	76.7	25	25	33.3
	S	73.3	15.7	23.3	75	75	66.7
Cefazolin	R	73.3	42.9	76.7	42.9	87.5	66.7
	S	26.7	57.1	23.3	57.1	12.5	33.3
Cefaclor	R	80	14.3	33.3	28.6	100	33.3
	S	20	85.7	66.7	71.4	0	66.7
Cefotaxime	R	16.7	0	0	12.5	87.5	0
	S	83.3	100	100	87.5	12.5	100
Ceftazidime	R	26.7	0	0	10	50	0
	S	73.3	100	100	90	50	100
Ceftriaxone	R	23.3	0	0	10	37.5	20
	S	76.7	100	100	90	62.5	80
Cefuroxime	R	73.3	42.9	33.3	28.6	87.5	86.7
	S	26.7	57.1	66.7	71.4	12.5	13.3
Ciprofloxacin	R	12	35.7	0	20.5	37.5	20
	S	88	64.3	100	79.5	62.5	80
Doxycycline	R	100	28.6	33.3	85.7	100	100
	S	0	71.4	66.7	14.3	0	0
Erythromycin	R	80	14.3	33.3	100	87.5	66.7
	S	20	85.7	66.7	0	12.5	33.3
Gentamycin	R	13.3	14.3	10	10	37.5	10
	S	86.7	85.7	90	90	62.5	90
Ofloxacin	R	0	28.6	0	10	50	13.3
	S	100	71.4	100	90	50	86.7
Tobramycin	R	6	0	0	20.5	0	20
	S	94	100	100	79.5	100	80

cefazolin, cefotaxime, cefuroxime and erythromycin. However amikacin, tobramycin, ceftriaxone, ciprofloxacin and gentamycin were found to be highly effective agents.

Similarly, wound isolates of *Proteus* species indicated high resistance to ampicillin, amoxicillin, erythromycin, doxycycline and moderate sensitivity to cefazolin, cefuroxime, cefaclor. However they were highly susceptible to cefotaxime, gentamycin, ciprofloxacin, tobramycin and amikacin.

Table.3 Percentage of resistance to all antibiotics among six isolated strains from wound samples

Antibiotics	% of resistance
Amikacin	0
Amoxicillin	86
Ampicilin	92
Azithromycin	45
Cefazolin	65
Cefaclor	48
Cefotaxime	20
Ceftazidime	15
Ceftriaxone	15
Cefuroxime	57
Ciprofloxacin	21
Doxycycline	75
Erythromycin	64
Gentamycin	16
Ofloxacin	17
Tobramycin	8

The *Klebsiella* **s**pecies isolated from wounds indicated 100% sensitivity to amikacin, ceftazidime, cefotaxime and moderate sensitivity to gentamycin, ofloxacin, ciprofloxacin, tobramycin and ceftriaxone. The species showed 100% resistance to ampicillin, amoxicillin and doxycycline. Table 2 shows the sensitivity and resistance pattern of isolates to different antibiotics. High level resistance is seen to ampicilin, amoxicillin and doxycycline. Amikacin is found to be very effective against all the isolates. Most of the isolates were sensitive to amikacin, ofloxacin, ciprofloxacin and gentamycin.

Wound is a major concern among healthcare practitioners, not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care, system (Bowler *et al*., 2001). In present study, rate of wound infection was high, that is similar to the other studies conducted in India (Suchitra and Lakshmidevi, 2009).

In the present study *Pseudomonas aeruginosa* (42.9%) and *Staphylococcus aureus* (24.3%) were the predominant organisms isolated from wound infections followed by other bacteria. A number of reports on wounds infection from different parts of the world indicated that both organisms were the most frequent isolates from different types of sepsis including wound (Mohammed *et al.,* 2011; Manjula *et al.*, 2007; Thanni *et al*., 2003 and Glacometti *et al*., 2000).

Staphylococcus epidermidis accounted for 15.7% 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 (Adebayor *et al.*, 2003). The lactose fermenters, made up of *Proteus* spp. (8.6%), *E. coli* (5.7%) *and Klebsiella* species (2.8%) isolates were encountered. This result is comparable with the findings of Mahmood (2000) who reported 26.13% enteric bacteria in their study.

Resistance to the selected antimicrobials was very high. The average resistance of the isolates to all the antibiotics was Gram positive cocci (27.9%) and Gram negative bacilli (46.4%). This is similar to the work of Andargachew *et al*., (2006) who reported that the overall multiple drug resistance patterns to be 58.5%. They also noted that the frequency of single as well as multiple drug resistance is alarmingly high.

As compared to other studies (Van Eldere, 2003), in our study *Pseudomonas aeruginosa* showed reduced sensitivity to commonly used antibiotics except amikacin (100%), ofloxacin (100%), ciprofloxacin (88%), gentamycin (86.7%), tobramycin (84%), cefotaxime (83.3%) and ceftriaxone (76.7). Ciprofloxacin and ofloxacin has been stated to be the most potent oral drug available for the treatment of *P. aeruginosa* infections. Similar reduced resistance of *P. aeruginosa* to ciprofloxacin has been reported in India (Raja and Singh, 2007). It is undoubtable that at the present time, the oral drug ciprofloxacin, ofloxacin and injection amikacin are the most effective antibiotics against *P. aeruginosa* involved in wound infection relative to most other commonly used drugs.

In the determination of the susceptibility of these *Staphylococcus aureus* on sixteen selected antibiotics by agar diffusion technique showed that *Staphylococcus aureus* tend to be resistant to a wider spectrum of antibiotics. This finding is in agreement with the work of Adcock *et al.*, (1998), Sani *et al.,* (2013) and CDC (1999) who reported that clinical Staphylococci are resistant to multiple antibiotics. In this study, *Staphylococcus epidermidis* was 100% sensitive to amikacin, ciprofloxacin, ofloxacin, tobramycin, ceftriaxone and ceftazidme, followed by gentamycin (90%).

In this study, 100% of the *E.coli* isolates were resistant to ampicillin, cefaclor, doxycycline and amoxicillin, 87.5% to erythromycin, cefuroxime, cefotaxime and cefazolin. Sensitivity pattern of *E.coli* in our study as compared to others were ciprofloxacin (97%), cefazolin (92%) (Weber *et al.,* 2009*),* ceftazidime (91%) ofloxacin (97%) (Kaufman *et al.*, 1998). So, reduced antibiotic sensitivity pattern noted for *E. coli* suggests its importance for hospital acquired infection.

Klebsiella pneumoniae was sensitive to amikacin, ceftazidime, cefotaxime (100%) followed by gentamycin (90%), ofloxacin (86.7%) and ciprofloxacin, tobramycin, ceftriaxone (80%). However, the previous study (Kaufman *et al.*, 1998) had shown reduced sensitivity to ciprofloxacin (63%), cefazolin (44.7%), ceftazidime (36.8%), and cefuroxime (34.2%)

Proteus mirabilis was sensitive to amikacin (100%), gentamycin, ceftazidime, ofloxacion (90%) ciprofloxacin (79.5%) and tobramycin

(79.5%). As compared to previous studies the sensitivity pattern were reduced for ciprofloxacin (75%), cefazolin (37.50%), (Mordi and Momoh, 2009), ceftazidime (37.50%), cefuroxime (25%) and ampicillin (95%).

There is an alarming increase of infections caused by antibiotic-resistant bacteria. Lack of uniform antibiotic policy and indiscriminate use of antibiotics may have lead to emergence of resistant bacterial strains. Particularly pseudomonas resistances to third generation antibiotics are real threat to control hospital acquired infection. In our study oral drugs ofloxacin, ciprofloxacin, injectable drugs amikacin, gentamycin and tobramycin shows good sensitivity against gram negative organisms. In addition, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management. This study suggests that if one could not wait the culture results in wound infection, ampicillin, amoxicillin, doxycycline, cefaclor and erythromycin are quite ineffective to treat these infections.

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