

Original Research Article

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Microbiological Profile of Wound Infections and their Antimicrobial Susceptibility Pattern in a Peripheral Tertiary Care Hospital of Eastern India

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ABSTRACT

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The main aim of this study to evaluate the bacterial etiology of wound infections and their antimicrobial susceptibility pattern. A total of 225 consecutive, non-repeating clinical specimens from various surface wounds were collected following inclusion and exclusion criteria and processed in the Microbiology laboratory from January to June 2012, following standard methodologies. A total of 186 bacterial isolates from 142 culture positive samples which consisted of 80 gram-negative bacilli, 102 gram-positive organisms, four atypical *Mycobacteria* and four *Candida albicans* were recovered. *Staphylococcus aureus* was the commonest isolate (41.93%) followed by *Escherichia coli* (11.82%) and *Pseudomonas aeruginosa* (9.67%) and coagulase negative *Staphylococcus* (7.53%). Methicillin resistance in *Staphylococcus aureus* was found to be 35.9% and all gram positive isolates showed cent percent sensitivity to vancomycin. For gram-negative bacilli, amikacin was most effective drug while β -lactam drugs and ciprofloxacin showed least sensitivity. Polymyxin B emerged as most sensitive agents against *Pseudomonas aeruginosa* and *Acinetobacter spp.* The antimicrobial susceptibility pattern suggested that drug therapy for wound infections should always be guided by the sensitivity reports for effective management of surface wound infections.

Introduction

Infections are common problems in surface wounds that occur as complications of trauma, surgical interventions, burn and secondary to invasive procedures. It is one of the key reasons for increased risks of patient morbidity and mortality. Bacteria seek to establish themselves in ecological niches to ensure survival and evolution. Open wounds are suitable bacteria to come and create their own niche (Stephan *et al.*, 2008). Source of wound infections include patients' own

commensal flora or organisms present in the nosocomial environment which are introduced to the patient by medical procedures or following surgery or trauma (Forbes *et al.*, 2002). Pattern of microorganisms causing wound infection is very often subject to a combination of biological, chemical and physical factors and every single organism has its specific demand which has to be satisfied for its long term survival in any given place. Surface wounds

seldom provide identical conditions. Hence, different wounds support different types of microorganisms to play (Cooper *et al.*, position document). In order of frequency, *Staphylococcus aureus* (*S. aureus*) accounts for 30-50% of skin and soft tissue infections followed by gram-negative bacteria, non-fermenters, *Streptococcus pyogenes* and anaerobes (Gales *et al.*, 2000). Resistance to antimicrobial drugs is increasing everyday at an alarming rate amongst both gram-positive and gram-negative bacteria. Clinically relevant bacteria have gradually developed not only single but also multi-drug resistance - A legacy of past decades of antimicrobial usage and misuse or abuse. Development of resistance in *S. aureus* to antimicrobial agents is a major issue, starting with resistance to penicillin G due to β -lactamase production followed by development of methicillin resistance in the 1960s by the *mecA* gene (Hartman *et al.*, 1984). Moreover, unlike blood stream infections, surface wound infection can become polymicrobial many a times. Relevance of isolation of the bacteria/organism as offender or mere colonizer must have to be discerned to restrict antimicrobial abuse. Atypical mycobacteria have now become a well known pathogen for surface wound infections, which may not respond to empirical therapy and lead to chronicity. Continuous surveillance is therefore mandatory to monitor the bacteriological profile and antimicrobial resistance pattern to guide antibacterial therapy. This study was conducted to find out microbiological spectrum of surface wound infections and their antimicrobial susceptibility pattern in a tertiary care hospital to guide rational antimicrobial therapy.

Materials and Methods

This prospective study included consecutive 220 pus, wound swab, exudate samples obtained from various types of wounds of the

patients attending Burdwan Medical College and Hospital between the months of January to June of year 2012. Samples from suspected or diagnosed cases of venous and decubitus ulcers were excluded. Two samples were obtained from each case before administration of an antibiotic or antiseptic dressing. One sample was subjected to Gram-staining and Ziehl Neelsen (ZN) staining examination by preparation of smear for presumptive identification of likely organism/s present. Another sample was used for conventional culture. While collecting pus or exudate from abscesses, wounds or other sites, special care was taken to avoid contamination from commensal organisms of skin as far as practicable. Each sample was inoculated on 5% Sheep blood agar, MacConky agar plates and Saboraud's Dextrose Agar slant. They were then incubated at 37⁰C for 18 to 24 hours. Suspected colonies were identified on the basis of morphological characteristics by Gram staining and by conventional biochemical tests following standard methodologies (Cruickshank *et al.*, 1980). All the identified bacteriological pathogens were subjected to antimicrobial susceptibility testing (AST) by Kirby Bauer Disc diffusion method recommended following guideline laid down by CLSI (2012). Standard strains of *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for the standardization of AST. Detection of Methicillin resistance was carried out by standard screening procedure using 30 μ g Cefoxitin disc obtained from Hi Media Laboratory, Mumbai. Methicillin resistance for *S. aureus* was considered present when a zone of inhibition was \leq 24 mm, and for coagulase negative *Staphylococcus* when the same was found to be \leq 23 mm (CLSI, 2012). Few samples showed growth of fungal yeasts in all three media used for study. The growths were further processed by Gram staining and germ tube testing (GTT) to establish their

identity. Samples from port site infections or surgical site infections were additionally inoculated onto Lowenstein Jensen media slant (in house prepared) and incubated at 37°C for up to eight weeks before disposal. Any growth appearing onto the slant were processed by Ziehl Neelsen staining examination and MPT64 protein antigen detection test (MPT64 antigen detection kit obtained from commercial manufacturer) for confirmation of their identity as atypical mycobacteria. MPT64 is one of the 24 KDa culture filtrate proteins (Yamaguchi *et al.*, 1989) (Nagai *et al.*, 1991) encoded by the RD2 region genes of *M. tuberculosis* complex organisms; and has been shown to be a specific antigen that differentiates the *M. tuberculosis* complex from the mycobacteria other than tuberculosis (MOTT) species (Hasegawa *et al.*, 2002), (Abe *et al.*, 1999). No AST was performed for the fungal yeasts and acid fast organisms isolated.

Results and Discussion

Out of 225 surface wound samples processed in this study, 142 (63.11%) were culture positive and 78 (34.66%) samples yielded no growth on aerobic culture. Five samples showed growth of *Diphtheroids* and aerobic spore bearing bacilli and they were considered contaminants, therefore not processed further. The 142 culture positive samples yielded 186 bacterial isolates out of which 108 had single pathogen and 17 had two pathogens each. Mixed growth of *S. aureus* and *E. coli* were present in nine; *S. aureus* and *Acintobacter spp.* in four out of these 17 samples.

Proteus vulgaris and *Enterococci* as mixed infections were found in four cases. Gram-positive bacteria constituted 106 out of 186 and rest 80 were Gram negative bacilli. *S. aureus* (n=78; 41.93%) was the commonest organism present in samples; followed by *E. coli* (n=22, 11.82%), *Pseudomonas*

aeruginosa (n=18, 9.67%) and Coagulase negative *Staphylococcus* (n=12, 8.55%) (Table 1). Antimicrobial susceptibility and resistance pattern of bacterial isolates to various antibiotics has been shown in table 2 and table 3. All four fungal yeasts were detected as *Candida albicans* by GTT. Acid fast organisms were detected as and upto Atypical Mycobacteria only by MPT-64 antigen testing. AST was not performed for atypical mycobacteria and *Candida spp.*

Infections by multi drug resistant bacteria are a global problem. The increasing incidence and prevalence of multi-drug resistant (MDR) organisms with limited treatment options such as MDR gram-negative bacilli, methicillin resistant *S. aureus* (MRSA), Vancomycin resistant *Enterococci* (VRE) both in community and hospital acquired infections are matter of concern. The milieu of multi drug resistance holds true for any site of the body including wound infections. *Staphylococcus aureus*, *E. Coli*, *Pseudomonas* and *Klebsiellae* were the main pathogens detected in this study.

Comparable organism profile was also observed by Zarger *et al.*, (2000) in their study with surgical site infections. Authors have observed a 41.93% prevalence rate of MRSA, which is almost close to what has been reported by Goyal (37.3%) (Goyal *et al.*, 2002); Rajadurai pandi (31.3%) (Rajadurai pandi *et al.*, 2006). Most strains of *Staphylococcus aureus* were resistant to Penicillin G (78.2%), Ciprofloxacin (71.79%), Erythromycin (76.92%) in the present study (Table 2). High resistance to these drugs has also been reported by some other Indian workers (Udai Shankar *et al.*, 1995) as well as from abroad (Bradley *et al.*, 1985). For *S. aureus*, low resistance was observed against amikacin (29.48%), Clindamycin (23.07%) and levofloxacin (46.15%).

Table.1 Frequency of isolates from the wound samples

Name of the isolate	Number of isolate (Total number of isolates; n =186)	Percentage (%)
<i>Staphylococcus aureus</i>	78	41.93
<i>E. coli</i>	22	11.82
<i>Pseudomonas aeruginosa</i>	18	9.67
Coagulase negative <i>Staphylococcus</i>	12	6.45
<i>Klebsiella spp</i>	09	4.83
<i>Enterobacter spp.</i>	08	4.30
<i>Citrobacter spp.</i>	07	3.76
<i>Proteus mirabilis</i>	06	3.22
<i>Proteus vulgaris</i>	06	3.22
<i>Acinetobacter spp</i>	06	3.22
<i>Candida albicans</i>	05	2.68
<i>Enterococcus spp.</i>	04	2.15
<i>Atypical Mycobacteria</i>	04	2.15

Table.2 Antimicrobial resistance pattern of gram positive bacterial isolates

Antimicrobial agent (in µg)	<i>Staphylococcus aureus</i> [number of isolate] (n=78, 41.93%)	CoNS [number of isolate] (n=12, 6.45%)	<i>Enterococcus spp</i> [number of isolate] (n=04, 2.15%)
Penicillin G	61(78.2 %)	7(58.33%)	4(100%)
Ampicillin	--	--	3(75%)
Cefoxitin	46(58.97%)	5(41.66%)	--
Ciprofloxacin	56(71.79%)	9(75 %)	4(100 %)
Levofloxacin	36(46.15%)	7(58.33%)	3(75 %)
Gentamicin	30(38.46%)	4(33.33%)	--
High level Gentamicin (120mcg)	--	--	2(50 %)
Erythromycin	60(76.92%)	12(100 %)	--
Clindamycin	18(23.07%)	3(25%)	--
Vancomycin	00	00	00
Teicoplanin	00	00	00
Linezolid	00	00	00
Amikacin	23(29.48%)	2(16.66%)	--

Table.3 Antimicrobial resistance pattern of gram-negative bacterial isolates

Antimicrobial agents	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>Klebsiella spp</i>	<i>Enterobacter spp.</i>	<i>Citrobacter spp.</i>	<i>P. mirabilis</i>	<i>P. vulgaris</i>	<i>Acinetobacter spp.</i>
(in µg)	n=22 (%)	n=18 (%)	n=8(%)	n=8(%)	n=7(%)	n=6(%)	n=6 (%)	n=6(%)
Amikacin	00	02 (11.11)	00	00	00	00	00	01 (16.66)
Gentamicin	03 (13.6)	02 (11.11)	02(25)	02(25)	00	00	00	02 (33.33)
Netilmycin	02 (9.09)	01 (4.54)	02(25)	02(25)	00	00	00	02 (33.33)
Cefpodoxime	20 (90.9)	--	08(100)	08(100)	04(57.14)	04 (66.66)	03 (50)	--
Cefotaxime	20 (90.9)	--	08(100)	08(100)	04(57.14)	04 (66.66)	03 (50)	06(100)
Ceftazidime	20 (90.9)	06 (33.33)	08(100)	08(100)	04(57.14)	04 (66.66)	03 (50)	06(100)
Aztreoname	20 (90.9)	03 (13.63)	08(100)	08(100)	04(57.14)	04 (66.66)	03 (50)	04 (66.66)
Piperacillin-tazobactam	10 (45.45)	05 (27.77)	06(75)	05(62.5)	03(42.85)	02 (33.33)	01 (16.66)	04 (66.66)
Cefepime	18 (81.81)	04 (18.18)	07 (87.5)	06(75)	05(71.42)	06 (100)	04 (66.66)	05 (83.33)
Imipenem	16 (72.72)	8(44.44)	6(75)	6(75)	4(57.14)	4(66.66)	4(66.66)	4(66.66)
Ciprofloxacin	16 (72.72)	14 (63.63)	06 (75)	07(87.5)	04(57.14)	02 (33.33)	00	04 (66.66)
Levofloxacin	08 (36.36)	14 (63.63)	06(75)	06(75)	04(57.14)	01 (16.66)	00	02 (33.33)
Co-trimoxazole	05 (22.72)	--	03(37.5)	02(25)	03(42.85)	03(50)	02 (33.33)	04 (66.66)
Polymyxin B	--	00	--	--	--	--	--	00

A sizable section of Coagulase negative *Staphylococci* (CoNS) were also resistant to methicillin (41.66%). But all *S. aureus* and CoNS strains were found to be 100% sensitive to vancomycin, linezolid, teicoplanin. Vancomycin is the drug of choice for treating infections caused by MRSA. However, this antibiotic should be used judiciously to prevent emergence of resistant strains among staphylococci. Isolation of *Enterococci* both in pure and mixed culture has been observed in four cases. Two of them were diabetic foot ulcer wounds and occurrence of *Enterococci* in them might be colonization. Hence those two samples were repeated twice one week apart without any invasive treatment. Both the samples yielded similar bacterial profile on culture. However, the *Enterococci* isolates have been found to be fully resistant to penicillin G (100%), ciprofloxacin (100%). A high rate of resistance has been observed by this organism towards ampicillin (75%), levofloxacin (75%) and High level Gentamicin (50%). All were susceptible to vancomycin, linezolid and teicoplanin. A very high rate of antibiotic resistance was seen with Gram negative bacterial isolates (Table 3). In the cases with *E. coli*, *Klebsiella spp*, *Enterobacter spp*, *Citrobacter spp*. and *Proteus spp*. resistance against amikacin were comparatively lesser or minimum or even nil in some instances. So amikacin can still be used for the treatment of these infections. For *Pseudomonas aeruginosa* polymyxin B (zero resistance), netilmycin, amikacin and gentamicin emerged as the most sensitive drugs. They showed moderate degree of resistance towards β -lactam drugs like ceftazidime, piperacillin – tazobactam. Fluoroquinolone drugs were not found to be very effective as found in the study for *Pseudomonas spp*. Organisms other than *Pseudomonas*, *E.coli* and *Klebsiella* have also shown high percentage of resistance against fluoroquinolone drugs; challenging the reliability of them as empirical therapeutic

options. All organisms including *Acinetobacter spp*. showed fairly low resistance against amikacin. It was not very difficult to choose a proper antibiotic for the cases infected with *Acinetobacter spp*, if the options of beta-lactam, co-trimoxazole and fluoroquinolone were excluded. This was a new finding as *Acinetobacter spp*. was notoriously known as multidrug resistant. Some variations in resistance pattern have been observed from place to place. Hence, similar study done by Shampa A has some differences in observation than ours (Shampa *et al.*, 2006). Atypical mycobacteria were looked for in all the samples received, additionally. They were detected in four cases (2.15%) out of total samples. All the samples were obtained from suspected SSI and PSI; and they were isolated as pure growth without any occurrence of mixed growth. Antimicrobial susceptibility pattern in the present study suggested that drug therapy for wound infections should always be guided by the sensitivity report. Infected wounds, which do not respond to treatment, should be evaluated for the presence of multi drug resistant organisms. Continuous surveillance is necessary to monitor antimicrobial resistance and antimicrobial policy should be updated according to the changing susceptibility pattern of bacteria.

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