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Research Article

PREVALENCE OF PATHOGENIC BACTERIAL ISOLATES IN WOUND INFECTION AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN: A STUDY CONDUCTED AT BURDWAN MEDICAL COLLEGE AND HOSPITAL, INDIA

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ABSTRACT

Background: Wound infections continue to be problematic in clinical practice where empiric treatment of infections is routine. These infections play an important role in development of chronicity consequently delaying wound healing.

Objectives: For identification of bacterial pathogens present in infected wounds and for determination of their antimicrobial susceptibility pattern from patients with pus and/or wound discharge.

Methods: A prospective study was conducted at Burdwan Medical College and Hospital over a period of three months (from March to May, 2017). Wound swab samples were collected from each study participant and inoculated onto appropriate media. The bacterial pathogens were identified using standard microbiological methods, Vitek 2 (*bioMérieux*) GN-ID card and 16S rRNA gene sequencing carried out by ABI 3500 Genetic Analyzer. Antimicrobial susceptibility tests were performed using disk diffusion technique following Kirby-Bauer method. Methicillin resistance in staphylococci was determined by cefoxitin disk diffusion (DD) test. ESBL was detected by phenotypic confirmatory disk diffusion test (PCDDT) using ceftazidime alone and in combination with clavulanic acid. MBL detection was done by imipenem-EDTA combined disk diffusion test (CDDT).

Results: Out of total 153 specimens 114 bacterial isolates (38.6% Gram positive isolates and 61.4% Gram negative isolates) were recovered showing an isolation rate of 74.5%. *Klebsiella pneumoniae* were the most frequently isolated bacteria accounting for 27(23.7%). Polymicrobial infection was found in 8(7%) of the infected wounds and was mainly constituted by two species. Moreover, 8 MRSA isolates and 3 ESBL and MBL producing Enterobacteriaceae isolates were obtained. 52.6% of bacterial isolates had MAR index > 0.2.Out of them 38(63.3%) were MDR isolates.

Conclusions: Prevalence of bacterial infections reached in high amount showing *Klebsiella pneumoniae* the most dominant one. As multidrug resistance among bacterial population is a major threat in recent years, so a constant and careful worldwide surveillance for them is urgently warranted.

INTRODUCTION

A wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue (Dionigi *et al.*, 2001). Although wounds can be of traumatic, non-traumatic or surgical types in accordance with their nature but the most common underlying event for all wound is trauma that may be accidental or intentionally induced (Taiwo *et al.*, 2002). Wound infections are the most common nosocomial infection (Leaper *et al.*, 1998).

Since colonization of wound is most frequently polymicrobial that are potentially pathogenic, any wound at some risk of becoming infected (Dai *et al.*, 2010). For convenience of microbial colonization, proliferation and infection wounds contribute to a moist, warm and nutritive environment resulting in pus formation in addition to general or local features of sepsis such as pyrexia, pain and induration (Fauci *et al.*, 2008; Shittu *et al.*, 2002; Bowler *et al.*, 2001; Cooper, 2005).Besides bacteria several pathogenic fungi, protozoa and viruses can cause wound infection (Taye *et al.*, 2011).

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The development of wound infection depends on interplay of many factors including old age; repeated trauma; blood perfusion; integrity and protective function of the skin; number and type of organism and their synergy; pathogenicity and virulence of bacterial species; use of antibiotics and immunocompetency of host. When virulence factors are expressed by one or more microorganisms they outcompete host's natural immune system. During vigorous toxin production immune cells become stimulated tending to cause local necrosis and disruption of delicate balance of critical mediators cytokines and proteases that are required for healing progression. Inspite of colonizing all chronic wounds low level of bacteria can benefit wounds by increasing amount of neutrophills, monocytes and macrophages thus improving prostaglandin E2 level and collagen formation (Sule *et al.*, 2002; Anupurba *et al.*, 2006; Shittu *et al.*, 2003; Weledji, 2012; Cogen *et al.*, 2008; Dryden, 2010).

Despite the progress made with respect to infection control and management wound infection still remains a serious and significant clinical challenge due to emergence of resistant bacterial pathogens for widespread and prolonged use of antibiotics that contribute to greater extent of MRSA (Methicillin Resistant *Staphylococcus aureus*). Although MRSA is a major healthcare-associated (HA-MRSA) as well as community-associated (CA-MRSA) infection causing a wide range of diseases including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles but in India this is one of the common causes of hospital-acquired infections (Vidhani *et al.*, 2001; Durai *et al.*, 2010; Mera *et al.*, 2011).

Enterobacteriaceae are another important cause of nosocomial and community acquired infections today. Among them ESBL (Extended spectrum β lactamase) and MBL (Metallo β lactamase) producing members have become a major concern in developing country like India due to their resistance to third generation cephalosporin and carbapenem (Coque *et al.*, 2008). These β lactamases which are responsible for widespread β lactam resistance hydrolyse amide bond of four membered characteristic β lactam ring thus rendering the antimicrobial ineffective (Prashant *et al.*, 2011). In addition to β lactam antibiotics ESBL producing isolates also exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluroquinolones (Dinesh *et al.*, 2011). Metallo β lactamases (MBLs) belonging to Amber's class B hydrolyse a wide variety of β lamtams including penicillin, cephems and carbapenems except aztreonam (Nishio *et al.*, 2004). MBLs require Zn for their activity and their Zn containing active sites are inactivated by metal chelators EDTA (Crowder *et al.*, 2006; Drawz *et al.*, 2010).

Knowledge of causative agents of wound infection has proved to be helpful in selection of empirical therapy,on infection control measures in health institution and in formulating rationales of antibiotic policy (Shittu *et al.*, 2002; Bowler *et al.*, 2001; Cooper, 2005; Taye *et al.*, 2011; Sule *et al.*, 2002; Anupurba *et al.*, 2006; Shittu *et al*, 2003). It is therefore important to identify antimicrobial resistant pathogens in wound infection. Our study was designed to determine the bacterial etiologies and their antimicrobial susceptibility pattern among patients with wound infection attending Burdwan Medical College and Hospital, India.

MATERIALS AND METHODS

Study Design and Period

This was a prospective study conducted at Burdwan Medical College and Hospital spanning 3 months from March to May, 2017.

Sample Collection

A total of 153 wound swab samples were collected from consecutive patients seen both inpatient and outpatient departments. Wounds were cleaned with normal saline and discharge was aseptically collected using sterile cotton swabs contained in test tubes from each study participant. Each sample tube was labeled carefully with patient's name, age, sex, date and transported immediately to bacteriology laboratory of Department of Microbiology at Burdwan Medical College for investigation and specimens were registered. Surgical wounds were inspected during first dressing and weekly thereafter till discharge.

Isolation and Identification

The collected samples were inoculated onto freshly prepared Nutrient ager, MacConkey agar and Blood agar, DNase test agar, Mannitol salt agar and incubated at 37°C aerobically for 24 to 48 hours. After obtaining pure colonies, further identifications were performed by standard microbiological technique including Gram staining, colony morphology and biochemical tests. Identification of bacterial isolates to the species level was done by Vitek 2 (*bioMérieux*) GN-ID card and 16s rRNA gene sequencing carried out by ABI 3500 Genetic Analyzer.

Antimicrobial Susceptibility Testing of Various Isolates

Antimicrobial susceptibility testing was carried out on Muller Hinton agar (MHA) plates by Kirby-Bauer disk diffusion method using commercially available antibiotic disks (HiMedia Labs, India) as recommended by Clinical and Laboratory Standard Institute (CLSI). The following antibiotics were tested by disk diffusion method : vancomycin (30 µg), erythromycin (30 µg), linezolid (30 µg), cefoxitin (30 µg), amoxicillin (10 µg), amikacin (30 µg), imipenem (10 µg), ceftazidime (30 µg), cotrimoxazole (25 µg), ceftriaxone (30 µg), polymyxin B (30 µg), ciprofloxacin (5 µg), piperacillin/tazobactam (10 µg), cefuroxime (30 µg). After inoculating test organism on a dry sterile MHA plate antibiotic disks were aseptically placed at appropriate equidistance and allowed to stand at 37°C for 24 hours. Diameter of zone of inhibition produced by each antibiotic disk was measured, compared with the guidelines and recorded after incubation (CLSI, 2013).

Determination of Multiple Antibiotic Resistance (MAR) index

Multiple Antibiotic Resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Krumperman, 1983).

Multidrug Resistance (MDR)

MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial classes (Magiorakos *et al*, 2012).

Detection of ESBL

This was performed by phenotypic confirmatory test (PCDT) as per the recommendations of CLSI. The ceftazidime (30 µg) disk alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 µg disk) were used.

Interpretation

An increase of ≥ 5 mm in zone of inhibition of the combination disks in comparison to the ceftazidime disk alone was considered to be ESBL producer.

Detection of MBL

This was performed by imipenem-EDTA combined disk method (CDT) as described by (Yong *et al.*, 2002). A lawn culture of test isolates was prepared. After allowing it to dry for five minutes, two imipenem disks, one with 0.5 M EDTA and the other a plain imipenem (10 µg) disk, were placed on the surface of agar plates approximately 30mm apart. The plates were incubated overnight at 37°C.

Interpretation

An increase in zone diameter of ≥ 7mm around imipenem + EDTA disk in comparison to imipenem disk alone indicated production of MBL.

Data Analysis

Statistical analysis of the data was carried out using SPSS 20. Association between type of wound and type of bacteria isolated and age of the patients and incidence of wound infection were analysed using $\chi 2$ test. P value ≤ 0.05 was considered statistically significant.

Ethical Consideration

Ethical clearance was obtained from the ethical committee of Burdwan Medical College and Hospital. Written informed consent was obtained from all study participants. The assent of children (< 18 years old) was obtained from their family or guardian. The laboratory results from the study participants were communicated to their doctors for appropriate treatment.

RESULTS

Prevalence of Wound Infection

Overall 153 specimens were collected from patients with clinical evidence of infection from March to May, 2017.

Table 1 Age distribution of patients with significant bacterial growth attending Burdwan Medical College and Hospital from March to May, 2017.

Age group	Total number of samples	Number of infected samples		P value
		Male	Female	
0 to 10	19	8	5	
11 to 20	35	20	7	
21 to 30	30	17	4	
31 to 40	25	15	4	
41 to 50	21	13	3	
51 to 60	11	5	4	
61 to 70	10	5	2	0.7
>70	2	2	0	
Total	153	85	29	

There were 107 male subjects and 46 female subjects. Ages ranged from 2.5 years to 84 years with a mean of 30.53 years (Table 1).

Of the 153 study participants (86 inpatients and 28 outpatients) a total of 114 (74.5%) samples yielded significant bacterial growth indicating wound infection while 39 samples were bacteriologically sterile.

Type of Wound

The most prevalent wound type was surgical (51.8%), followed by trauma (29.8%) and then was non-traumatic causes (18.4%). There was significant association between type of wound and the type of bacteria isolated (p = 0.01) (Table 2).

Table 2 Bacterial isolates of wound infection among inpatients and outpatients attending Burdwan medical college and Hospital from March to May, 2017.

Bacterial isolates	Inpatients n (%)		Outpatients n (%)		Frequency n (%)
	Male	Female	Male	Female	
Klebsiella pneumoniae	13 (22.4)	7 (25)	5 (25)	2 (25)	27 (23.7)
Klebsiella oxytoca	2 (3.4)	2 (7.1)	-	-	4 (3.5)
E. coli	9 (16)	4 (14.3)	2 (10)	4 (50)	19 (16.7)
Citrobacter sp.	1 (1.7)	2 (7.1)	2 (10)	-	5 (4.4)
Acinetobacter sp.	2 (3.4)	1 (3.5)	-	-	3 (2.6)
Acinetobacter baumannii	-	-	1 (5)	-	1 (0.9)
Pseudomonas sp.	4 (6.8)	-	-	-	4 (3.5)
Pseudomonas fluorescens	-	-	1 (5)	-	1 (0.9)
Proteus mirabilis	2 (3.4)	-	-	-	2 (1.8)
Bordetella trematum	1 (1.7)	-	1 (5)	-	2 (1.8)
Morganella morganii	1 (1.7)	-	-	-	1 (0.9)
Salmonella typhi	1 (1.7)	-	-	-	1 (0.9)
MSSA	4 (6.8)	3 (11)	3 (15)	1 (12.5)	11 (9.6)
MRSA	5 (8.6)	2 (7.1)	1 (5)	-	8 (7)
CoNS	10 (17.2)	6 (21.4)	2 (10)	-	18 (15.7)
Enterococcus sp.	3 (5.2)	1 (3.5)	2 (10)	1 (12.5)	7 (6.1)
Total	58 (100)	28 (100)	20 (100)	8 (100)	114 (100)

Key:- MRSA: Methicillin resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus;* CoNS: Coagulase negative staphylococci; -: Zero.

Age

There was greater incidence of wound infection in the 11 to 20 year age but no significant association was observed between age of patients and the incidence of wound infection (p = 0.7) (Table 1).

Bacterial Etiologic Agents Isolated from Wounds

Gram negative bacterial species were commonly isolated, 70 (31.4%) verses Gram positive bacterial species, 44 (38.6%). The presence of only one species isolated from each sample was the most frequent 106 (93%). 8 (7%) of the wound swab cultures showed polymicrobial growth (Figure 1).

Klebsiella pneumoniae were the most prevalent bacteria isolated from wound swabs accounting for 27 (23.7%) followed by *E. coli* and *Staphylococcus aureus* (each 19;16.7%); CoNS (18;15.7%); *Enterococcus* sp. (7;6.1%); *Citrobacter* sp. (5;4.4%); *Klebsiella oxytoca* and *Pseudomonas* sp. *(*each 4;3.5%); *Acinetobacter* sp. (3;2.6%), *Proteus mirabilis* and *Bordetella trematum* (each 2;1.8%); *Pseudomonas fluorescens, Acinetobacter baumannii, Morganella morganii and Salmonella typhi* (each 1, 0.9%) (Table 2).

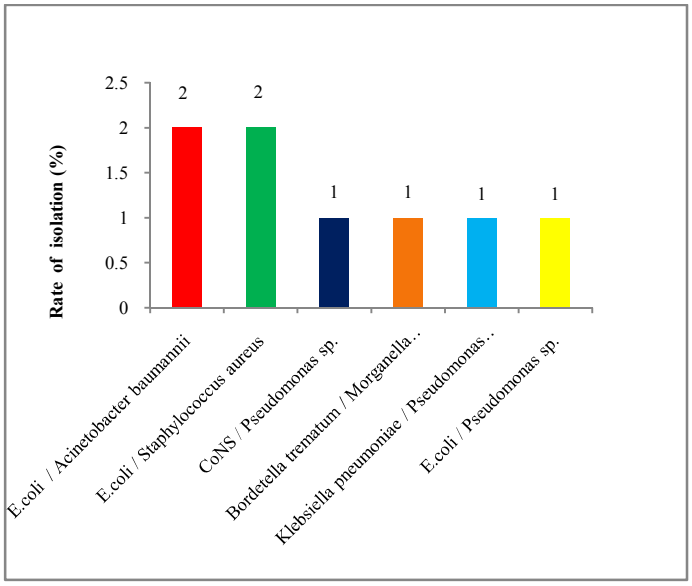

Figure 1 Percentage of polymicrobial infection of patients with infected wounds attending Burdwan Medical College and Hospital from March to May, 2017.

Table 3 Frequency of bacterial isolates of wound infection according to wound types

Bacterial isolates	Type of wound			P value
	Surgical	Traumatic	Non-traumatic	
Klebsiella pneumoniae	20	6	1	
Klebsiella oxytoca	3	0	1	
E . coli	13	2	4	
Citrobacter sp.	3	1	1	
Acinetobacter sp.	2	1	0	
Acinetobacter baumannii	0	1	0	
Pseudomonas sp.	1	2	1	
Pseudomonas fluorescens	0	1	0	
Proteus mirabilis	0	2	0	0.01
Bordetella trematum	0	1	1	
Morganella morganii	0	1	0	
Salmonella typhi	1	0	0	
MSSA	2	7	2	
MRSA	5	0	3	
CoNS	7	8	3	
Enterococcus sp.	2	1	4	
Total	59	34	21	

Key:- MRSA: Methicillin resistant Staphylococcus aureus, MSSA: Methicillin sensitive staphylococcus aureus, CoNS: Coagulase negative staphylococci

Antimicrobial Susceptibility Pattern of Bacterial Isolates from Wound Swab Cultures

Antimicrobial susceptibility pattern of Gram positive and Gram negative bacterial isolates were presented in Table 4 and 5 respectively. The predominant isolate *Klebsiella pneumoniae* revealed high level of sensitivity to all of the antibiotics tested.

8 (7%) Methicillin resistant *Staphylococcus aureus* (MRSA) isolates were obtained. All of them were highly resistant to cefoxitin. Furthermore, 3 ESBL and MBL producing Enterobacteriaceae isolates were found. None of the isolates showed coexistence of ESBL and MBL in the same isolate.

MAR (Multiple Antibiotic Resistance) Index and Multidrug Resistant (MDR) Isolates

Multiple Antibiotic Resistance (MAR) index distribution among bacterial isolates was shown in Table 6. Proportion of isolates with MAR index > 0.2 was 52.6% while those had MAR index of ≤ 0.2 was 47.4%. We have found 38(33.3%) MDR (Multidrug Resistant) isolates.

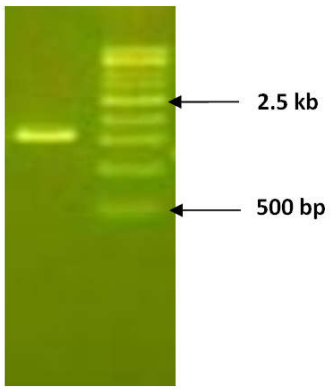

Figure 2 PCR amplification of 16S rRNA gene from bacterial isolate (Size of PCR amplified product is ~ 1.5 kb).

Table 4 Antimicrobial susceptibility pattern of Gram positive bacteria isolated from wound swab cultures of patients attending Burdwan Medical College and Hospital from March to May, 2017

Bacterial isolates	Antimicrobial agents (%)							
	Pattern	AMK	VAN	ERY	LNZ	CX	AMX	CIP
Staphylococcus aureus	R	4 (21.1)	-	10(52.6)	-	8(42.1)	12(63.2)	5(26.3)
(n = 19)	S	15(78.9)	19(100)	9(47.4)	19(100)	11(57.9)	7(36.8)	14(73.7)
CoNS	R	4 (22.2)	1(5.6)	8(44.4)	-	2(11.1)	9(50)	5(27.8)
(n = 18)	S	14(77.8)	17(94.4)	10(55.6)	18(100)	16(88.9)	9(50)	13(72.2)
Enterococcus sp.	R	2(28.6)	-	3(42.9)	-	Nt	4(57.1)	3(42.9)
(n = 7)	S	5(71.4)	7(100)	4(57.1)	7(100)		3(42.9)	4(57.1)
Total	R	10(22.7)	1(2.3)	21(47.7)	-	10(22.7)	25(56.8)	13(29.5)
(n =44)	S	34(77.3)	43(97.7)	23(52.3)	44(100)	27(61.4)	19(43.2)	31(70.5)

Key:- S: Sensitive; R: Resistant; Nt: Not tested; -: Zero; n: number of isolate; AMK: Amikacin; VAN: Vancomycin; ERY: Erythromycin; LNZ: Linezolid; CX: Cefoxitin; AMX: Amoxicillin; CIP: Ciprofloxacin; CoNS: Coagulase negative staphylococci.

Table 5 Antimicrobial susceptibility pattern of Gram negative bacteria isolated from wound swab cultures of patients attending Burdwan Medical College and Hospital from March to May, 2017

Bacterial isolates	Pattern	Antimicrobial agents (%)									
		AMK	AMX	COT	CAZ	CTR	CIP	PTZ	IMP	PB	CXM
Klebsiella pneumoniae (n = 27)	R	9(33.3)	14(51.9)	19(70.4)	8(29.6)	7(25.9)	17(63)	2(7.4)	9(33.3)	-	13(48.1)
	S	18(66.7)	13(48.1)	8(29.6)	7(25.9)	5(18.5)	10(37)	7(25.9)	18(66.7)	2(7.4)	14(51.9)
Klebsiella oxytoca (n = 4)	R	1(25)	2(50)	2(50)	1(25)	Nt	1(25)	-	1(25)	Nt	1(25)
	S	3(75)	2(50)	2(50)	3(75)		3(75)	1(25)	3(75)		3(75)
E. coli (n = 19)	R	3(15.8)	10(52.6)	9(47.4)	4(21.1)	2(10.5)	4(21.1)	2(10.5)	3(15.8)	-	5(26.3)
	S	16(84.2)	9(47.4)	10(52.6)	11(57.9)	2(10.5)	15(78.9)	1(5.3)	16(84.2)	2(10.5)	14(73.7)
Citrobacter sp. (n = 5)	R	1(20)	3(60)	3(60)	-	2(40)	2(40)	-	1(20)	Nt	2(40)
	S	4(80)	2(40)	2(40)	3(60)	-	3(60)	1(100)	4(80)		3(60)
Acinetobacter sp. (n = 3)	R	-	-	-	-	-	-	Nt	-	Nt	-
	S	3(100)	3(100)	3(100)	2(100)	1(100)	3(100)		3(100)		3(100)
Acinetobacter baumannii (n = 1)	R	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	-	1(100)	-	1(100)
	S	-	-	-	-	-	-	1(100)	-	1(100)	-
Pseudomonas sp. (n = 4)	R	1(25)	2(50)	2(50)	1(25)	1(25)	2(50)	1(25)	1(25)	-	2(50)
	S	3(75)	2(50)	2(50)	2(75)	-	2(50)	-	3(75)	1(25)	2(50)
Pseudomonas fluorescens (n =1)	R	1(100)	1(100)	1(100)	Nt	1(100)	1(100)	Nt	-	-	Nt
	S	-	-	-		-	-		1(100)	1(100)	
Proteus mirabilis (n = 2)	R	-	-	-	-	Nt	-	Nt	-	Nt	-
	S	2(100)	2(100)	2(100)	2(100)		2(100)		2(100)		2(100)
Bordetella trematum (n = 2)	R	-	1(50)	2(100)	2(100)	2(100)	1(50)	-	-	Nt	Nt
	S	2(100)	1(50)	-	-	-	1(50)	2(100)	2(100)		
Morganella morganii (n = 1)	R	-	1(100)	1(100)	Nt	-	-	Nt	-	Nt	1(100)
	S	1(100)	-	-		1(100)	1(100)		1(100)		-
Salmonella typhi (n =1)	R	1(100)	1(100)	1(100)	Nt	1(100)	1(100)	-	1(100)	Nt	1(100)
	S	-	-	-		-	-	1(100)	-		-
Total (n = 70)	R	18(25.7)	36(51.4)	41(58.6)	17(24.3)	17(24.3)	30(42.9)	5(7.1)	17(24.3)	-	26(37.1)
	S	52(74.3)	34(48.6)	29(41.4)	30(42.9)	9(12.9)	40 (57.1)	14(20)	53(75.7)	7(10)	41(58.6)

Key:- S: Sensitive; R: Resistant; Nt: Not tested; -: Zero; n: number of isolate; AMK: Amikacin; AMX: Amoxicillin; COT: Cotrimoxazole; CAZ: Ceftazidime; CTR: Ceftriaxone; CIP: Ciprofloxacin; PTZ: Piperacillin/Tazobactam; IMP: Imipenem; PB: Polymyxin B; CXM: Cefuroxime.

Table 6 Distribution of Multiple Antibiotic Resistance (MAR) index among bacteria isolated from patients with infected wounds attending Burdwan Medical College and Hospital from March to May, 2017

Bacterial Isolates	MAR index										
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Klebsiella pneumoniae	8	2	-	2	1	-	1	4	-	9	-
Klebsiella oxytoca	2	-	-	1	-	-	-	-	-	1	-
E. coli	9	1	-	3	1	-	1	1		3	-
Citrobacter sp.	2	-	-	1	-	-	-	1	1	-	-
Acinetobacter sp.	3	-	-	-	-	-	-	-	-	-	-
Acinetobacter baumannii	-	-	-	-	-	-	-	-	1	-	-
Pseudomonas sp.	2	-	-	-	-	-	-	1	1	-	-
Pseudomonas fluorescens	-	-	-	-	-	-	-	1	-	-	-
Proteus mirabilis	2	-	-	-	-	-	-	-	-	-	-
Bordetella trematum	-	-	-	-	-	2	-	-	-	-	-
Morganella morganii	-	-	-	-	1	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-	-	-	1	-
Staphylococcus aureus	7	2	-	2	3	-	1	4	-	-	-
CoNS	9	1	-	3	1	-	2	1	-	1	-
Enterococcus sp.	3	-	1	-	-	1	-	2	-	-	-

Key: - : zero CoNS: Coagulase negative staphylococci

DISCUSSION

Wound infection is a major concern among healthcare practitioners who share a goal for prevention of infection in charge of wound management.

Our study demonstrated a high prevalence (74.5%) of pathogenic bacteria in wounds that is in agreement with similar study in East Africa (Azene *et al*., 2011) but different from another study in Nigeria reporting a prevalence of 86.1% (Kemebradikumo *et al.*, 2013).

We found association between type of wound and type of bacteria isolated that is consistent with some studies done in Nigeria (Okesola *et al.*, 2011; Otokunefor *et al.*, 1980). All swabs from surgical wounds yielded significant bacterial growth in our study and were thus deemed to indicate infection. Ideally, the age of a patient seems likely to have a bearing on wound infection people at extremes of life being more prone to this infection. However, we observed no association between age of patients and incidence of wound infection a finding that is inconsistent with the results of a study done in Niger Delta region (Egbe *et al.*, 2011) and another in Ethiopia (Azene *et al.*, 2011).

In our study the majority of the wounds were colonised with a single bacterial species and *Klebsiella pneumoniae* were the predominant isolate that is consistent with a study in Western Nigeria (Taye *et al.*, 2011) but different from other studies in Nigeria reporting *Staphylococcus aureus* to be predominant (Leaper *et al.*, 1998; Shittu *et al.*, 2002; Egbe *et al.*, 2011). Only 7% of the wounds displayed polymicrobial infection where bacterial synergy enhances their survival, therefore hampering infection eradication. Moreover, as microbial biofilm is considered to be a complication for successful antibiotic treatment so researchers are seeking for new alternative therapies useful to enhance wound healing such as laser therapy (Kirketerp *et al.*, 2008; Baffoni *et al.*, 2012).

The bacterial isolates were examined for their susceptibility pattern to most commonly used antibiotics in therapy. Despite increasing concern about antibiotic resistant bacteria appropriate use of systemic antibiotics is still recommended where there is clear evidence of infection (Howell *et al.*, 2005; NICE, 2004; EWMA, 2006). The resistance to cefoxitin is particularly important as it can give us percentage of MRSA; in our study a relevant percentage (42.1%) of *Staphylococcus aureus* was methicillin resistant. MRSA are major nosocomial pathogen causing significant morbidity and mortality. In India significance of MRSA had been recognized relatively late and epidemic strains of these MRSA are usually resistant to several antibiotics (Durai *et al.*, 2010).

In addition the incidence of infections caused by β lactam resistant Enterobacteriaceae has increased in recent years due to production of various enzymes. Among them ESBL (Extended spectrum β lactamase) and MBL (Metallo β lactamase) producing members constitute a serious threat to current β lactam therapy that leads to treatment failure (Wadekar *et al.*, 2013).Our study showed ESBL and MBL production in 3 Enterobacteriaceae isolates. Out of them 2 were *Klebsiella pneumoniae* and 1 was *E. coli.* The early detection of β lactamase producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such isolates (Wadekar *et al.*, 2013).

MAR index > 0.2 has been said to be an indication of isolates originating from an environment where antibiotics were often used (Krumperman, 1983). Analysis of MAR index of bacterial isolates in our study showed that 52.6% had MAR index > 0.2. This findings reflect that a greater proportion of the isolates are likely to be form high risk source and originate from an environment where several antibiotics are used. Out of 60 multiple antibiotic resistant isolates 38(63.3%) were MDR isolates. Multi drug resistant isolates (MDR) were defined as those which depicted resistance to > 3 classes of antimicrobial tested (Magiorakos *et al.*, 2012). As emergence of multidrug resistant bacterial strains is a growing concern in effective management of wound infection so proper monitoring and optimization of antibiotic use is required.

CONCLUSIONS

As severe antimicrobial resistance in wound infection was observed among patients attending Burdwan Medical College and Hospital, India so a serious and urgent intervention was needed to stem the spread and further evolution of this resistance. We also advocate the inclusion of anaerobic culture in routine microbiology culture investigation. Furthermore, a combined interaction and cooperation between microbiologists, clinicians and infection control team is recommended.

Additional points

Limitation of the study

Due to resource limitation we were unable to characterize each of the isolate at their species level like *Acinetobacter sp., Pseudomonas sp., Enterococcus sp., Citrobacter sp*. and were also unable to perform MIC (Minimum Inhibitory Concentration). Inspite of having importance anaerobic bacteria culture was not done for a variety of reasons, the main one lacking equipment and funds.

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