Antibiotic Susceptibility Pattern of Bacterial Isolates Causing Wound Infection Among the Patients Visiting B & B Hospital

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Abstract

A wound is any physical injury involving a break in the skin, and exposed subcutaneous tissues provide a favorable substratum for a wide variety of microorganisms to contaminate and colonize. In this study a total of 870 plus samples were collected from patients visiting B & B hospital suspecting wound infection and then analyzed. The causative agents were isolated, identified by culture and biochemical tests and their susceptibility pattern to antibiotics were determined by using CLSI guidelines. Out of total samples, 476(44.8%) showed bacterial growth. Among growth cases 22.9% were mixed growths. Among all bacterial isolates, 12 species were identified of them, 70.6% were Gram-negative and 29.4% were Gram-positive. Among Gram-positive isolates, *Staphylococcus. aureus* (74.2%) was most common followed by CoNS (8.6%), Non haemolytic *Streptococci* (8.6%), *Enterococcus* spp. (4.3%) and β -haemolytic *Streptococci* (4.3%). Among Gram-negative bacteria, the most common isolate was *Pseudomonas aeruginosa* (31.5%) followed by *E.coli* (24.8%), *Acinetobacter* spp. (14.6%), *Enterobacter* spp. (14.0%) and *Klebsiella* spp. (13.4%). *Proteus* spp. and *Citrobacter* spp. were less common.With regard to AST pattern, *S. aureus* was most susceptible to chloramphenicol and highly resistance to penicillin. Among them, 7.7% were MRSA. For other Gram-positive isolates as well, effective drug was found to be chloramphenicol. For *P. aeruginosa*, the most effective drug was imipenem (94.3%) followed by amikacin (63.2%). Likewise, for other Gram-negative bacteria most effective drug was imipenem followedby amikacin. Among the total *P. aeruginosa* isolates, 58.5% were found to be MDR. For all these *P. aeruginosa* isolates MIC on gentamycin and ciprofloxacin illustrate the simultaneous presence of ciprofloxacin resistance and reduced gentamycin susceptibility. These results indicated that antibiotic resistance in Gram-positive and Gram-negative bacteria were increasing in alarming trend that leads to the failure of treatment.

Key words: wound infection, multidrug resistannce (MDR), minimum inhibitory concentration (MIC)

Introduction

A wound is a breach in the skin and the exposure of subcutaneous tissue following loss of skin integrity providing moist, warm and nutritive environment that is conducive to microbial colonization and proliferation (Bowler *et al*. 2001). Wound can be infected by a variety of microorganisms ranging from bacteria to fungi and parasites as well as virus (Church *et al*. 2006). The most common organisms are *Staphylococcus aureus,*, *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* species and *Acinetobacter* species. (Gupta *et al*. 2002, Eselbelahie *et al*. 2013). The control of wound infections has become more

challenging due to widespread bacterial resistance to antibiotics, and to a greater incidence of infections caused by methicillin-resistance *S. aureus,*, polymicrobic flora (Shittu *et al.* 2002).

The present study was conducted to determine the prevalence of MDR *P. aeruginosa*, antibiotic susceptibility pattern of isolates and MIC for *P. aeruginosa* to provide guidelines on suitable treatment and ensure infection-control policies leading to appropriate treatment of wound infection and prevention of emergence of antimicrobial resistance.

Methodology

A exploratory study was carried out at the Microbiology Laboratory of B & B Hospital, Lalitpur. Required samples (wound Pus/swab) obtained from the patient visiting B and B hospital were received in the laboratory within 30 minutes of collection. The samples were collected following aseptic technique which then were macroscopically analysed for their color and odor and microscopically analysed for type and morphology of bacteria. The samples were inoculated on blood agar (BA), Mac-conkey agar (MA), chocolate agar (CA) and mannitol salt agar (MSA) using standard protocol (Isenberg 2004). Mac-conkey agar plates were incubated aerobically, while BA and CA plates were incubated in microaerophilic condition at 37^0C for 24 to 48 hours in incubator. All the isolates were identified by colony morphology, staining reaction and the biochemical properties (Cheesbrough 2006). The antimicrobial susceptibility test of isolates was performed by Modified Kirby Bauer disc diffusion method using the standard guidelines and interpretive criteria of the CLSI (2013). After this isolated *S. aureus* was screened for methicillin resistance using cefoxitin disc (30μg) as per standard guidelines provided by CLSI, (2013). And MDR *P. aeruginosa* was identified as resistant to ≥3 antimicrobial classes (Magiorakos *et al.* 2011). MICs of ciprofloxacin and gentamycin were determined by agar dilution method (EUCAST 2000, Andrews 2001) and the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2013). The tests were performed by making a series of antibiotic concentrations on Mueller–Hinton agar plates. A reference strains, *E.coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as control. All the data were entered in SPSS version 20 and WHONET 5.6. Statistical analyses were done using the same software.

Results and Discussion

Of 870 sample processed, 390 (44.1%) showed their growth. Among growth positive cases, 304(77.9%) were monoisolates while 86(22.1%) had polymicrobial growth. (56%). High rate of growth positive rate in infection was found in case of male gender than female gender, i.e. 46.9% in male and 37.4% in female, which was found to be statistically significant (p-value < 0.05). Among total growth, the highest growth rate was found in age group 21-30 (25.4%), followed by 31-40 (16.2%) and 11-20 (16.2%). Least growth was found in an age group of 0-10 and above 60 years. A regard to the location of patients, high growth was found in indoor patients (54.9%) than outdoor (OPD) patients (38.8%) and emergency patients (20.3%).

Out of 476 growth positive wound swab samples, Gram negative bacteria were found 70.6%, while Gram positive bacteria in 29.4%. Among the gram negative isolates, *P. aeruginosa* 106(31.5%) was the most predominant followed by *E.coli* 82(24.4%), *Acinetobacter* spp. 49(14.6%). *Enterobacter* spp. 47(14.0%) and *Klebsiella* Spp 45(13.4%). Other bacteria like *Proteus* spp. 6(1.8%) and *Citrobacter* Spp 1 (0.3%) were lower in a number. Similarly, among Gram positive bacteria, *S. aureus* 104 (74.2%) was the most common isolates followed by CoNS 12 (8.6%), NHS 12 (8.6%) *Enterococcus* spp. and β Haemolytic *Streptococci*.

Among 104 *S. aureus*, the resistance pattern showed that the most effective antibiotics were chloramphenicol (4.8%) followed by clindamycin (7.7%) and cefoxitin(7.7%) while highly resistant to Penicillin(99.1%) and amoxicillin(86.5%), (Table 1).

Table 1. Antimicrobial susceptibility pattern of the *Staphylococcus aureus*

Antibiotics	Resistance	Total
Ciprofloxacin	42 (40.4%)	104
Ofloxacin	41(39.4%)	104
Gentamycin	42(40.4%)	104
Ceftriaxone	13(22.1%)	104
Chloramphenicol	5(4.8%)	104
Cefoperazone/Sul	10(9.6%)	104
Penicillin	103(99.1%)	104
Amoxicillin	90(86.5%)	104
Erythromycin	35(33.6%)	104
Cefoxitin	8(7.7%)	104
Clindamycin	8(7.7%)	104

P. aeruginosa

Among 106 *P. aeruginosa* isolates, imipenem (5.7%) was found to be most effective followed by amikacin (36.8%) and highly resistant against the ceftazidime (73.6%), (Table 2).

Table 2. Antimicrobial susceptibility pattern of *P. aeruginosa*

| Antibiotics	Resistance	Total
Ciproloxacin	55(51.9%)	106
Ofloxacin	55(51.9%)	106
Gentamycin	57(53.8%)	106
Amikacin	39(36.8%)	106
Ceftazidime	78(73.6%)	106
Chloramphenicol	63(59.4%)	106
Cefoperazone/Sul	48(45.3%)	106
Meropenem	55(51.9%)	106
Imipenem	6(5.7%)	106
Piperacillin/Taz	56(52.8%)	106

E. coli

Among 82 E.coli isolates, imipenem (3.6%) was found the most effective drug followed by amikacin (13.4%) and highly resistant against ciprofloxacin (54.9%) and ofloxacin (54.9%), (Table 3).

Table 3. Antimicrobial susceptibility pattern of the *E. coli.*

| Antibiotics	Resistance	Total
Ciproloxacin	45(54.9%)	82
Ofloxacin	45(54.9%)	82
Gentamycin	23(28.1%)	82
Amikacin	11(13.4%)	82
Ceftriaxone	56(68.3%)	82
Chloramphenicol	17(20.7%)	82
Cefoperazone/Sul	14(17.1%)	82
Meropenem	11(13.4%)	82
Imipenem	3(3.6%)	82
Piperacillin/Taz	24(29.3%)	82

Prevalence of MDR *P. aeruginosa*

Among the total *P. aeruginosa* isolates, 62 were found to multi drug resistance, of which 2 were resistant to 3 antimicrobial classes. Among MDR isolates, 7 were resistant to three antimicrobial classes; 4 were resistant to 4 classes; 12 were resistant to 5 antimicrobial classes; 6 were resistant to 6 antimicrobial classes and 33 were resistant to 7 antimicrobial classes.
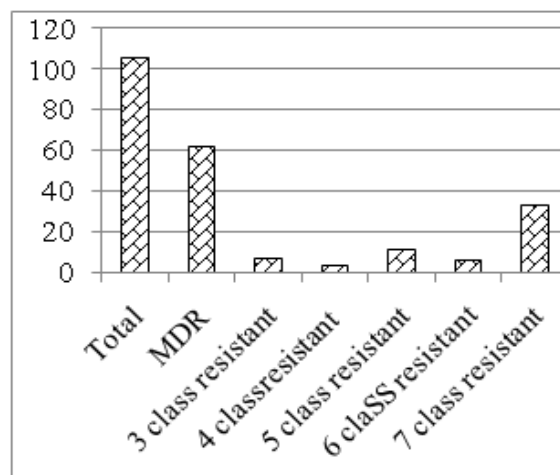
Fig.1. MDR antimicrobial class resistant of *P. aeruginosa*.

MIC on gentamycin and ciprofloxacin of *P. aeruginosa*
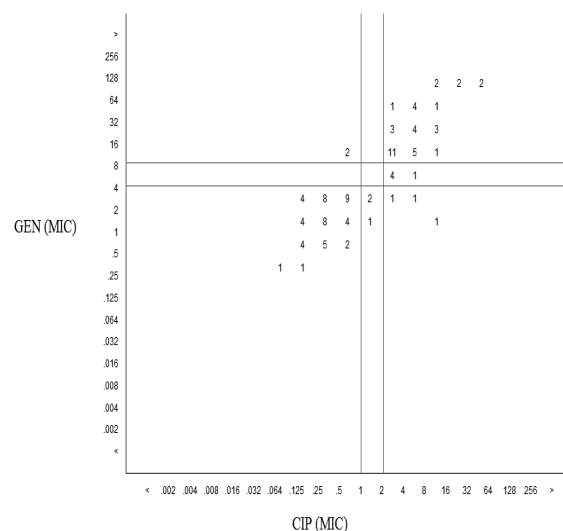
Fig. 2. MIC of *P. aeruginosa* (gentamycin vs ciprfloxacin)

In our study, out of 870 samples from patients with wound infection, 44.9 % samples showed positive growth. In similar studies conducted by Karki (2011) in pediatric cases, Shrestha and Basnet (2009) showed growth positive were 47% & 50% respectively. A study conducted by Giacometti *et al.* (2000), Manyahi (2012) in the tertiary hospital, more than 90% show growth. Culture negative results could be due to non infective bacteria, manual error in collection, transportation, culture media and collection of sample from patient taking antibiotic.

Among growth cases, 77.9% cases showed single isolates and 22.1% cases showed multiple isolates. The result agreed with the study carried out by Fadeyibi *et al.,* (2012) and Manyahi (2012). The growth was found to be higher in male patients 46.9% than in female patients (37.4%). Similar study carried out by Parajuli (1997) agree with our result. The relatively higher percentage of male patients might be due to active involvement of males in outdoor activities, including agricultural work resulting into high infection and prevalence of high rate of accidental cases among them (Naik & Deshpande 2011).

Among total growth of 44.9%, the highest growth rate was found in age group 21-30 (25.4%), followed by 31-40 (16.2%) and 11-20 (16.2%). Least growth was found in an age group of 0-10 and above 60 years. Our findings agree with the studies carried out by Kumari (2008) and Akinjogunla *et al.* (2009). The reason behind this age group (21-30) showed high growth among all age categories may be due to high prevalence of this age group in our study as well as the group being active age group or working age group hence the chances of occurring accidents leading to hospital admission.

The high rate of growth was found to be in indoor patients (54.9%) than OPD patients (38.8%) and emergency patients (20.3%), which was similar to the study of Iregbu *et al.* (2013). The reason for the high prevalence in ward may be due to factor associated with acquisition of nosocomial pathogens in patients with recurrent long term hospitalization, complicating illness, prior administration of antimicrobial agents.

In our present study, out of total bacterial isolates, 70.6% were Gram negative and 29.4% were Gram-positive bacteria. In a similar study conducted by Giri *et al.* (2008), Iregbu *et al.* (2013), Eselbelhie *et al.* (2013) Gram negative bacteria were found predominant. Most of the hospital-based studies nowadays show that Gram-negative bacteria was more prevalent than Gram-positive bacteria. Banjara *et al.*(2002) at TUTH showed that high rate of Gram-negative bacteria are found in HAI.

Among these isolated pathogens *P. aeruginosa* being the most predominant (22.3%) occurring in total isolates and 31.5% of Gram-negative bacteria while *S. aureus* was the second predominant isolate among the total isolates. In a similar study conducted by Thanni *et al.* (2003), *P. aeruginosa* was most predominant one (29.9%) among the total isolates while *S. aureus* was predominant (27.5%) among Gram positive isolates. The study conducted by Iregbu *et al.* (2013) gram-negative bacilli constituted 66% of all the pathogens with *P. aeruginosa* as the most frequent (19%). Higher prevalence of *S. aureus* among Gram-positive bacteria and 2nd predominant isolates among the total, seen in this study was also reported by other researchers, Mehta *et al.* 2000 and Mohammad *et al.* 2013. Many researchers said that most commonly isolated. *S. aureus* was ubiquitous and the most common cause of localized suppurative lesions in human beings.

With regard to antibiotic susceptibility pattern, the most effective antibiotic against *S. aureus* was chloramphenicol (4.8%) followed by clindamycin (7.7%), penicillin (99.1%) and amoxicillin (86.5%) was least effective. These results agree with Shrestha, (2009). Other developing countries, also reported high resistance rates of *S. aureus* to amoxicillin and penicillin (Anguju *et al.* 2007).

In a present study, 7.7 % of *S. aureus* isolates were MRSA, and emerged as a major pathogens worldwide. Studies on MRSA have shown their wide variation. Naik and Deshpande (2011) showed 8.0% of MRSA which agree to our study. Chen and Zhang 2007, at China showed (82.5%). *P. aeruginosa* was most effective against imipenem (5.7%) followed by amikacin (36.8%). The least effective antibiotic was ceftazidime (73.6%). This was agreed by Mahmood, 2000, towards amikacin (92%) followed by meropenem and imipenem (88%) sensitive. Rajput *et al* (2008), in which *Pseudomonas* isolated from burn wound was less resistant to imipinem (26%) and highly resistant to ceftazidime (70%). For *E.coli,* imipenem (3.6%), amikacin (13.4%) and meropenem (13.4%) were considerably effective while ceftriaxone (68.3%) and ciprofloxacin (54.9%) and ofloxacin (54.9%) were least effective. This was supported by Mahmood (2000) and where *E. coli* was found to be 100% sensitive to imipenem.

Resistance to antibiotics antimicrobial agents is a major concern worldwide and is exemplified by the global spread of MRSA, MDR *P. aeruginosa, Acinetobacter* spp. In our study out of 106 *P.*

aeruginosa isolates 58.5% were MDR. This result was supported by the studies conducted by Upadhya *et al*. 2012, Gupta *et al*. 2002, where MDR *P. aeruginosa* were 58.3 % & 54.8% respectively. The increase in a number of immune compromised hosts, debilitating effects of prolonged hospitalization, application of medical equipments (airways, catheters, etc.) and improper infection-control measures have contributed to the dissemination of MDR-bacteriain hospital settings (Esen & Leblebicioglu, 2008, Furtado *et al*. 2009). Resistance to antimicrobial agents is a major concern worldwide and High resistance pattern of *P. aeruginosa* is major problem. All the isolates subjected for determination of MIC values of commonly used drug i. e. fluoroquinolone (Ciprofloxacin) and aminoglycoside (Gentamycin). Scattergram correlating the MICs of gentamycin and ciprofloxacin illustrated the simultaneous presence of ciprofloxacin resistance and reduced gentamycin susceptibility.

In our study Gram-negative bacteria predominated over Gram-positive bacteria. The most common isolates were *P. aeruginosa S. aureus, E coli Klebsiella* spp. and *Acinetobacter* spp. Most of the organisms, particularly *P. aeruginosa, S. aureus* and *E.coli* spp. were isolated from inpatients location were resistant to most of the antibiotic tested. *S.aureus* isolates were resistant to penicillin and amoxicillin, whereas *P. aeruginosa,, Acinetobacter* spp., *E. coli* and *Klebsiella* spp. were resistant to ciprofloxacin, ofloxacin and resistance to third generation cephalosporin. These results indicate that antibiotic resistance pattern in Gram-positive and Gram-negative bacteria are increasing in alarming trend that leads to the failure of treatment.

Acknowledgments

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