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Prospective Study on Bacterial Isolates with their Antibiotic Susceptibility Pattern from Pus (Wound) Sample in Kathmandu Model Hospital

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ABSTRACT

Background: Wound infection is a very common infection throughout the world and is causing a great fear in developing countries like Nepal. The present study was carried out in Kathmandu Model Hospital with an aim to find out the bacteriological episodes in pus samples in relation to age and sex, Antibiotic Susceptibility Testing (AST) and drug resistance pattern among the isolates.

Methodology: A total of 157 pus samples included in the study were processed in the Microbiology laboratory of Kathmandu Model Hospital using standard microbiological techniques. Identification of organisms was done on the basis of microscopy, colony morphology, and biochemical tests. The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar and interpreted as per CLSI guidelines.

Results: Analysis of 157 pus samples showed (93, 59.24%) culture positive cases with (63, 67.74%) Multi Drug Resistance (MDR) isolates. Age group (21-30) was found to be the most vulnerable age group. *Staphylococcus aureus* (29, 28.71%) was the predominant organism isolated among gram positive bacteria and *Escherichia coli* (26, 25.74%) among gram negative bacteria. Gentamycin was the most sensitive antibiotic among gram positive bacteria whereas Amikacin being the most sensitive Antibiotic among gram negative bacteria.

Conclusion: Antibiograms of isolates can serve as a tool for physicians to start an empirical treatment and minimize the drug resistance problem.

Keywords: Antibiogram, Bacterial isolates, Antibiotic susceptibility, Pus, Kathmandu Model Hospital

BACKGROUND

Wound is defined as break in the normal continuity of the skin or a body structure caused by injury [1]. Wound infection can be defined as the presence of organisms in a wound that are multiplying and eluciting a host reaction. In laboratory terms, it is often defined as an organism counts of greater that 10^5 gram of tissue [2]. Surgical wound infection, acute soft tissue infection, bite wound infection, burn wound infection, pyogenic wound infection, leg and decubitus (pressure) ulcer infections are the categories of wound infection [3].

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Infection can occur at an incision site within 30 days of an operation, but wounds that are closed and primarily healed does not necessarily mean infected [4]. Pre-existing illness, length of operation, wound class, and wound contamination are the predisposing factor for wound infection [5]. Surgical wound and skin infections accounts for 70-80% mortality. It leads to almost one third of the hospital acquired infection among surgical patients [6].

People in Nepal are generally prone to agricultural wounds, traffic accidents and domestic injuries. People are not aware and knowledgeable about the prevention of injuries and disabilities. It may lead to complications due to poor management of wounds at the initial stage. Absence of facilities in the district and peripheral hospitals along with traditional unscientific household practices and

lack of safety system result in wound infection. In Nepal, MDR pathogens are more prevalent because of the imprudent use of antibiotics and people failing to take the full course of treatment. The important factors associated with resistant bacteria are poor resources for infection control, lack of manpower trained in controlling infection in hospital and poor hospital management system [7]. The present study was done to find the resistance pattern of isolates to different group of Antibiotics.

METHODOLOGY

This study was carried out from July 25, 2010 to October 26, 2010 in the Microbiology laboratory of Kathmandu Model Hospital thereby covering a total period of 3 months. A total of 157 pus samples were collected from the patients ranging in age from 3 months to 84 years. Pus samples were collected on a sterile cotton swab or aspirated in syringe and labeled with date, time, and the patient's name, age and sex. For laboratory investigation, two pus swabs were collected; one for the direct smears preparation and the other one for culture.

Macroscopic and microscopic observations were noted. Samples were inoculated on to Blood agar (BA), MacConkey agar (MA) and were incubated at 37°C for 24 hrs to 48 hrs in aerobic condition; the study did not include anaerobic bacteria culture.

After overnight incubation, the culture plates were examined for bacterial growth and identified using standard microbiological techniques which included colony characteristics, staining reactions and biochemical reactions such as catalase, coagulase, indole, methyl red, Voges- Proskauer, citrate, urease, Triple Sugar Iron Agar (TSI) and Oxidase test.

The antibiotic sensitivity testing of all isolates was performed by Kirby Bauer's disc diffusion method on Muller Hinton agar (MHA) and interpreted as per CLSI guidelines. Quality control is considered as one of the important factor for the correct result interpretation [8]. Standard strains taken for quality control was *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) [9].

RESULTS

Out of 157 pus samples collected, (89, 56.69%) were from male patients with (49, 52.69%) positive cases and (68, 43.31%) were from female patients with (44, 47.31%) positive cases. The samples were collected from different age group ranging from 3 months to 84 years. Age group 21-30 years was found to be most affected age group (17, 18.27%) (Table 1).

Table 1: Age and gender wise distribution of patient with positive cases (+ve: culture positive, %= percentage)

AGE GROUP (years)	GENDER				M+F	M+F (+VE)	M+F (%)	M+F (+VE %)
	MALE (M)		FEMALE(F)					
	No.	+ve	No.	+ve				
< 11	9	7	6	6	15	13	9.55	13.98
11-20	6	5	11	6	17	11	10.83	11.83
21-30	25	9	15	8	40	17	25.5	18.27
31-40	13	7	11	8	24	15	15.3	16.13
41-50	12	3	6	4	18	7	11.5	7.53
51-60	16	14	5	2	21	16	13.4	17.2
>60	8	4	14	10	22	14	14.01	15.05
Total	89	49	68	44	157	93	100	100

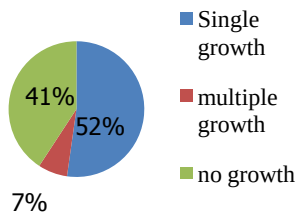

Fig. 1 Growth pattern of total Sample

Out of 157 pus samples, (93, 59.24%) was culture positive with single growth (82, 52.23%), multiple growths (11, 7.006%) and no growth (64, 40.76%) (Fig. 1).

101 bacterial isolates of 11 species were isolated which included 3 species of gram positive bacteria and 8 species of gram negative bacteria. (53, 52.48%) were gram positive isolates and (48, 47.52%) were gram negative isolates. The incidence of *Staphylococcus aureus* (29, 28.71%) was highest followed by *Escherichia coli* (26, 25.74%), Coagulase Negative Staphylococci (CoNS) (22, 21.78%), *Proteus mirabilis* (1, 0.99%), *Enterobacter aerogens* (1, 0.99%), and *Morganella morganii* (1, 0.99%) respectively.

Similarly, among 93 culture positive cases 63 MDR isolates was found. The incidence of MDR was found to be highest in *Escherichia coli* (21, 33.33%), followed by *Staphylococcus aureus* (16, 25.4%), CoNS (13, 20.63%), *Citrobacter freundii* (4, 6.35%), *Acinetobacter spp* (3, 4.76%), *Pseudomonas aeruginosa* (2, 3.17%), *Proteus mirabilis*, *Proteus vulgaris*, *Streptococcus Viridans*, *Enterobacter aerogens* each (1, 1.6%) respectively (Fig. 2).

For gram positive isolates, Gentamycin (43, 91.5%) was found to be most sensitive antibiotic followed by Cloxacillin (43, 81.13%), Erythromycin (39, 78%) while Cotrimoxazole (15, 28.30%), and Cephalexin (11, 20.75%) being the least sensitive antibiotic respectively (Table 2).

Table 2: Antibiotic susceptibility pattern of Gram positive isolates

ANTIBIOTICS	Sensitive		Intermediate		Resistance		No. od Antibiotics Used
	No.	%	No.	%	No.	%	
AMOXYCILLIN	24	45.28	0	0	29	54.7	53
CEPHOTAXIME	37	69.81	5	9.43	11	20.75	53
COTRIMOXAZOLE	15	28.3	3	5.66	35	66.03	53
CIPROFLOXACIN	41	77.36	0	0	12	22.64	53
CEPHALEXIN	11	20.75	1	1.88	41	77.36	53
CLOXACILLIN	43	81.13	0	0	10	18.87	53
ERYTHROMYCIN	39	78	0	0	11	22	50
GENTAMYCIN	43	91.5	1	2.13	3	6.4	47

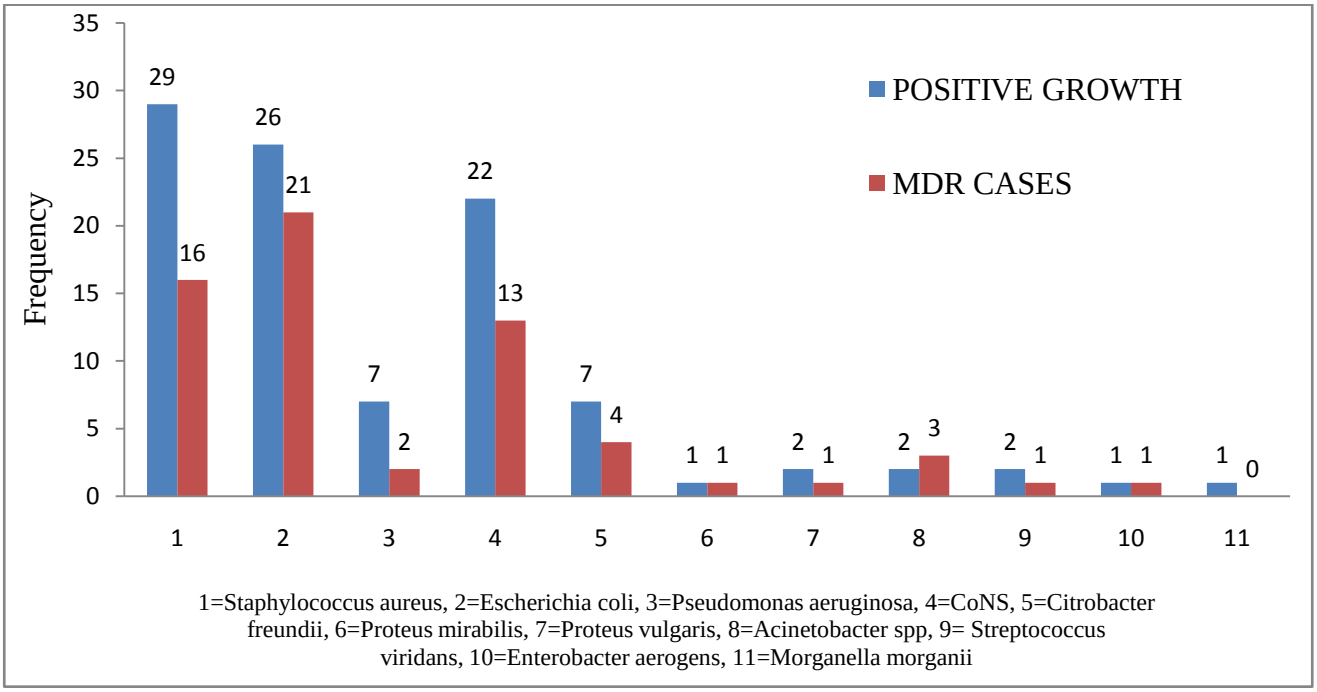

Fig. 2 Distribution of organisms isolated with MDR cases

For gram negative isolates, Amikacin (45, 93.75%) was found to be the most sensitive antibiotic followed by Gentamycin (42, 89.36%), Ciprofloxacin (27, 56.25%) while Amoxycillin (13, 32.5%) and Cotrimoxazole (14, 29.16%) being the least sensitive antibiotic respectively (Table 3).

Table 3: Antibiotic susceptibility pattern of Gram negative isolates

ANTIBIOTIC	Sensitive		Intermediate		Resistance		No. od Antibiotics Used
	No.	%	No.	%	No.	%	
AMOXYCILLIN	13	32.5	0	0	27	67.5	40
CEPHOTAXIME	19	39.5	2	4.2	27	56.25	48
COTRIMOXAZOLE	14	29.2	0	0	34	70.83	48
CIPROFLOXACIN	27	56.3	0	0	21	43.75	48
CEFIXIME	14	33.3	0	0	28	66.67	42
OFLOXACIN	3	50	0	0	3	50	6
GENTAMYCIN	42	89.4	0	0	5	10.64	47
AMIKACIN	45	93.8	0	0	3	6.3	48
CEFTRIAXONE	18	42.9	1	2.4	23	54.76	42
CEFTAZIDIME	16	45.7	1	2.4	18	51.43	35

For *Staphylococcus aureus*, Cloxacillin (100%) was the most sensitive antibiotic followed by Erythromycin (92.9%), Ciprofloxacin (89.7%) while Cotrimoxazole (24.14%) and Cephalexin (10.34%) being the least sensitive antibiotic respectively (Table 4).

Table 4: Antibiotic Susceptibility pattern for isolates of *S. aureus*

Antibiotic	Sensitive		Intermediate		Resistance		No. of Antibiotic Used
	No.	%	No.	%	No.	%	
Amoxycillin	10	34.5	0	0	19	65.5	29
Cephotaxime	21	72.4	4	13.8	4	13.8	29
Cotrimoxazole	7	24.1	3	10.3	19	65.5	29
Ciprofloxacin	26	89.7	0	0	3	10.3	29
Cephalexin	3	10.34	0	0	26	89.7	29
Cloxacillin	29	100	0	0	0	0	29
Erythromycin	26	92.9	0	0	2	7.1	28
Gentamycin	21	87.5	1	4.2	4	8.4	24

For *Escherichia* coli, Amikacin (100%) was the most sensitive antibiotic followed by Gentamycin (96%), Ciprofloxacin (42.3%), Ofloxacin (40%) while Amoxycillin (11.5%) and Ceftazidime (21.4%) being the least sensitive antibiotic respectively (Table 5).

Table 5: Antibiotic Susceptibility pattern for isolates of *Escherichia coli*

ANTIBIOTIC	Sensitive		Intermediate		Resistance		No. of Aantibiotic used
	No	%	No.	%	No.	%	
Amoxycillin	3	11.5	0	0	23	88.5	26
Cephotaxime	7	26.9	1	3.9	18	69.2	26
Cotrimoxazole	8	30.8	0	0	18	69.2	26
Ciprofloxacin	11	42.3	0	0	15	57.7	26
Cefixime	7	26.9	0	0	19	73.1	26
Ofloxacin	2	40	0	0	3	60	5
Gentamycin	24	96	0	0	1	4	25
Amikacin	26	100	0	0	0	0	26

DISCUSSION

Out of 157 pus samples processed, 93, 59.24% showed significant bacterial growth with single growth, (82, 52.23%), multiple growth (11, 7.006%) and no growth (64, 40.76%). 88, 56.1% were from male patients with (49, 52.69%) positive cases and (69, 43.9%) were from female patients with (44, 47.3%) positive cases. Our study agrees with a similar study carried out by Parajuli *et al* (1997), Shrestha *et* al (1997), Tuladhar *et al* (1997), Onche and O. Adedeji (2004), Karkee *et al*, (2008), Kumari *et al*, (2008), Shrestha *et al*, (2010). The relative higher cases in male may be due to their greater participation in outdoor activity.

The samples were collected from patients ranging in age from 3 months to 84 years with age group 21-30 years being the most affected age group which agrees with the studies done by researchers in Nepal, Parajuli *et al*, (1997),

Tuladhar *et al*, (1998), Karkee *et al*, (2008), and Kumari *et al*, (2008). Since the age group 21-30 are the most active age group and they are mostly involved in outdoor activity they might be the most affected age group.

Among the total 93 positive cases 101 organisms were isolated in which 53(52.48%) were gram positive bacteria and (48, 47.52%) were gram negative bacteria. Our study agrees with similar study done by parajuli *et al* (1997) and karkee *et al*, (2008). But study done by Zafar *et al*, (2007) showed that frequency of gram positive (54, 49.54%) and gram negative (55, 50.45%) was almost equal and the study carried out by Anbumani *et al* (2006), also found the equal presence of Gram positive cocci (49.6%) and Gram negative bacilli (49.5%). Our study disagrees with Dangol *et al*, (1995) and Kumari *et al*, (2008), their study showed that gram negative bacteria were isolated more compared to the gram positive bacteria. Shrestha *et al*, (2010) showed that Gram negative bacilli (52.3%) had a slightly higher frequency than Gram positive cocci.

A total of 11 different bacterial species were isolated from 101 isolates in which *Staphylococcus aureus* (29, 28.71%) was found to be the most predominant organism followed by *Escherichia coli* (26, 25.74%), CoNS (22, 21.78%), *Pseudomonas aeruginosa* (7, 6.93%), *Citrobacter freundii* (7, 6.93%), *Proteus vulgaris* (2, 1.98%), *Acinetobacter* species (2, 1.98%), *Streptococcus viridans* (2, 1.98%), *Proteus mirabilis* (1, 0.99%), *Enterobacter aerogens* (1, 0.99%), *Morganella morganii* (1, 0.99%). Our findings were almost similar to Karkee *et al*, (2008), Shrestha *et al*, (2009). *S. aureus* and *E. coli* was the predominant organism isolated among gram positive and gram negative bacteria respectively.

But, Banjara *et al*, (1998), Giacometti *et al*, (2000), Banjara *et al*, (2002), Ekrami *et al*, (2007), Zafer *et al*, (2007). On the other hand, the study done by Shrestha *et al*, (2010) showed that Coagulase Negative Staphylococci (CoNS) was the predominant organism isolated and *S. aureus* was found to be the third commonest organism isolated. Mordi *et al*, (2009) found that *Proteus* spp was the predominant organism isolated among gram negative bacteria.

Among 93 positive cases, (63, 67.74%) MDR isolates were found. The incidence of MDR was found to be highest in *Escherichia coli* (21, 33.33%), followed by *Staphylococcus aureus* (16, 25.4%), CoNS (13, 20.63%), *Citrobacter freundii* (4, 6.35%) respectively. Banjara *et al*, (2002) found that (93, 47.2%) MDR isolates were found among total 197 isolates isolated in which *E. coli* (26, 55.3%) dominated among the total MDR isolates isolated which was relatively higher from our finding. Similarly, Shrestha *et al*, (2010) found that (15, 34%) MDR isolates were found among 44 isolates with *E. coli* (3, 60%) dominating among the MDR isolates followed by CoNS and *C .freundii* respectively which was relatively higher from our finding.

In in-vitro antibiotic suscceptibility pattern of Gram positive organism, Gentamycin (43, 91.5%) was found to be the most sensitive followed by Cloxacillin (43, 81.13%), Erythromycin (39, 78%), Ciprofloxacin (41, 77.36%), Cephotaxime (37, 63.81%), Amoxycillin (24, 45.28%), Cotrimoxazole (15, 28.30%), Cephalexin (11, 20.75%). Tuladhar *et al*, (1998) also found that Gentamycin (89%) was the most sensitive antibiotic for gram positive organism and Ciprofloxacin (84%) being the second most sensitive antibiotic. Parajuli *et al*, (1997) found that Cephotaxime (90.62%) and Ciprofloxacin (90.14%) were the most effective antibiotic for the Gram positive bacteria which was different from our findings but it was found that in both the study Cephalexin was the most resistant antibiotic. Katuwal *et al*, (1998), found that for Gram positive cocci the most effective antibiotic was Ciprofloxacin (74.66%) followed by Cloxacillin (64%), and Gentamycin (61.1%). Similarly, karkee *et al*, (2008), kumari *et al*, (2008) study showed that Ofloxacin was the

most sensitive antibiotic and in case of kumari *et al*, (2008), Gentamycin was found to be the least sensitive antibiotic which was different from our findings. Shrestha *et al*, (2009) showed that Cloxacillin (98.53%) and Erythromycin (97.06%) were the most effective antibiotics for Gram positive bacteria and Amoxycillin (65.20%) was the least effective antibiotic.

In in-vitro Antibiotic susceptibility pattern of gram negative organism Amikacin (44, 93.62%) was found to be most sensitive antibiotic followed by Gentamycin (41, 89.1%), Ciprofloxacin (27, 57.45%), Cephotaxime (18, 38.3%) respectively which is similar to the study done by Shrestha *et al*, (2009) and dissimilar to the study done by Parajuli *et al*, (1997), Tuladhar *et al*, (1998), Katuwal *et al*, (1998).

In case of *S. aureus*, it was found that Cloxacillin (100%) was the most sensitive antibiotic followed by Erythromycin (92.9%), Gentamycin (87.5%) while Co-trimoxazole (24.14%) and Cephalexin (10.34%) being the least sensitive antibiotic respectively which is similar to the study done by Singh *et al*, (2006), cloxacillin (87%) followed by Erythromycin (81.8%). No any cases of MRSA were found which is markedly different from the studies done by Ekrami *et al*, (2007), 58%, Chia *et al*, (1993), 17.30%, Giacometti *et al*, (2000), 74.2% of MRSA. Similar study done by Shrestha *et al*, (2010), also found only one case of MRSA which showed that MRSA are comparatively in very low number.

In case of *E. coli* it was found that Amikacin (100%) was the most sensitive antibiotic followed by Gentamycin (96%), Ciprofloxacin (42.3%), Ofloxacin (40%) while Amoxycillin (88.46 %) was the most resistant followed by Cefixime (73.1 %) and Ceftazidime (71.43 %). Similar study done Singh *et al*, (2006), Shrestha *et al*, (2009) showed Amikacin (77.8% and 94.38%) was the most sensitive antibiotic for *E. coli* respectively.

CONCLUSION

Though wound infection is a non eradicable problem, but preventive measures, good disinfection and treatment protocols, clean surgical procedures, proper care of wounds and hygienic practices help to minimize the incidence of the wound infections. Frequent and timely conversation between the microbiologist and wound care practiconers also plays a major role in limiting the wound infection in hospitals. Similarly, the antimicrobial susceptibility testing result suggests that some antibiotics would have very limited usefulness for the prophylaxis or the empirical treatment of wound infection. The result might serve as a foundation for establishing empiric therapeutic approaches for the management of such infections in Kathmandu Model Hospital and other Health care institutions of Nepal.

COMPETING INTERESTS

We declare that we don't have competing of interest.

AUTHORS' CONTRIBUTIONS

PC, CS, SRP performed the laboratory experiments and data collection. SK, BS supervised and guided in the research. PC, BT performed statistical analysis, conceived part of this study and revised the manuscript. All authors read and approved the final manuscript.

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