Original Article

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Isolates and their Antibiogram in Different Samples from a Tertiary Care Hospital, Kathmandu.

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ABSTRACT

Introduction: The emergence and spread of antimicrobial resistance constitutes a major risk for human health by limiting the success of these agents in the therapy. 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. Knowledge of etiological agent and its sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies. The aim of this study was to prepare a local antibiogram of the commonly isolated organism at a tertiary care hospital.

Methods: A prospective study was conducted at KMC Teaching Hospital, from April 14th to 17th September 2014. Laboratory data of culture and sensitivity were collected from hospital Microbiology Laboratory and analyzed using SPSS software.

Results: In our study most of the isolates were Gram negative *with Klebsiella Spp.* and *Escherichia coli* being predominant with many MDR isolates. The isolates were found to be resistant to different groups of drugs. We found least resistance to Chloramphenicol, Imepenem and Amikacin. Most of the resistance was found against Amoxicillin and Erythromycin. Among gram positive *S. aureus* was predominant with 64.7% MRSA and 23.1% VRSA isolates.

Conclusions: The most sensitive drug for gram positive was Chloramphenicol, Cephotaxim and Norfloxacine. The only drug which was 100% sensitive to Gram Negative organism was Chloramphenicol.

Keyword: antibiotic susceptibility test; MRSA; VRSA; antibiotics.

INTRODUCTION

The emergence and spread of antimicrobial resistance constitutes a major risk for human health. Resistance to antibiotics limits the success of these agents in the therapy and prevention of infectious diseases[1-7]. 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[8]. Several intrinsic factors such as point mutation, gene amplification and extrinsic factors like horizontal transfer of resistant gene between bacteria within and across species by transposons, integrins or plasmids have been postulated for the development of resistance, which cannot be reduced once developed even by restricting the antibiotic

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usage[9]. Resistance based on decreased entry of drugs has been found for Penicillin, Cephalosporin, Amino glycosides and Tetracycline in the Enterobacteriaceae and Pseudomonasaeruginosa. Beta-lactams resistance has increased significantly being encountered in Enterobacteriaceae and *Pseudomonas species*[10]. Multidrug resistance by bacteria is a matter of concern. The definition of MDR bacteria has not been universally agreed on but generally denotes bacteria that are resistant to atleast three antibiotics of different classes[11].

Knowledge of etiological agents of infections and their sensitivities to available drugs is of immense value to the rational selection and use of antimicrobial agents and to the development of appropriate prescribing policies[12]. Keeping all these facts in view, the present study was carried out with aim to determine the bacterial isolates from different clinical samples and describe their antibiogram i.e. sensitivity and resistance patterns to different antibiotics, which would thus enable the determination of empiric antimicrobial strategies for the early treatment of imminent medical events.

METHODS

This was a prospective study carried out in the Department of Microbiology, Kathmandu Medical College Teaching Hospital, a centrally located tertiary care medical center in the Kathmandu valley, Nepal, from14th April 2014 to 17th Septmber 2014.

The samples included in our study were Pus swab, aspirate, Sputum, Stool, Throat swab, High Vaginal Swab, Central Venous Catheter Tip, Endotracheal Tube, Aspirates and Body Fluids like Ascitic fluid, Pleural fluid, Peretoneal fluid, Cerebro spinal fluid etc that were sent to microbiology lab for culture and sensitivity.

All samples like pus and/or wound discharge or other body fluids samples submitted at KMC Teaching Hospital during the study period were included in this study. Laboratory results were noted in the register of Hospital Microbiology Laboratory unit and the data was collected from there following a standard data collection format after checking the completeness of the data.

The samples were inoculated on to Chocolate Agar, Blood Agar and MacConkey Agar. Plates were incubated at 37°C for 24 hours (Chocolate Agar incubated in CO_2 enriched environment). Following incubation, isolated colonies obtained were identified by series of biochemical tests following standard procedures[13].

Antimicrobial susceptibility was determined by the Kirby- Bauer disc-diffusion method performed on Muller-Hinton Agar plates. Plates were incubated at 35-37°C for 24 hours. Antibiotics disc used in this study were Ciprofloxacin (5 µg), Ofloxacine (30 µg) Nalidixic Acid, Norfloxacin, Ceftriaxone (30 µg), Ceftazidime, Cefotaxime, Chloramphenicol (30 µg), Co-trimoxazole (25 µg), Amikacin, Gentamycin, Tobramycin, Tetracycline, Cloxacillin, Oxacillin, Linezoline,Vancomycin, Amoxicillin, Piperacillin/ Tazobactam, Erythromycin, Azithromycin, Imepenem, Nitrofurantoin, Cefepime and Novobiosin (Hi Media Laboratory Ltd, Mumbai, India). The zones of inhibition were measured and result interpreted according to the CLSI guidelines[14].

Detection of VRSA was done by observing the zone size around Vancomycin.

Detection of MRSA was done by using Oxacillin Disc on the bacterial Lawn Culture of *S. aureus*. After overnight incubation, the zone of Inhibition was measured. An inhibition zone diameter less than or equal to 10 mm was considered as MRSA[13].

Data were cleaned manually and entered into and analyzed by using SPSS Statistic Version 20.0 software.

RESULTS

Out of total 847 samples received, we found growth in 247 samples (29.17 %). Out of 247 positive samples, 128 (51.8%) were identified as Gram Negative Organism whereas 119 (48.2%) were identified as Gram Positive Organism (Figure 1).

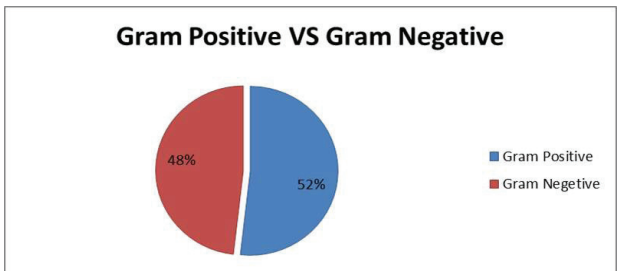

Figure 1. Gram positive verses negative percentage

The highest of number of growth was seen from Pus (59.5%) followed by Sputum (21.1%) and Aspirate (6.5%).The percentage isolates from different clinical samples are shown in Table 1.

Table 1. Isolates from different samples.

Sample	Positive	Percentage
Aspirate	16	6.5
Catheter Tip	10	4
CSF	2	0.8
Drain Tip	2	0.8
ET Tube	7	2.8
Foly's Catheter Tip	4	1.6
HVS	5	2.0
Pus	147	59.5
Sputum	52	21.1
Stool	1	0.4
Throat swab	1	0.4
Total	247	100

Our study revealed that within Gram Negative Organism all isolates were Bacilli/Coco Bacilli. *Klebsiella Spp.*and *E. coli* were predominant (39.8% and 35.9% respectively) followed by *Acinetobacter Spp.* (14.8%), *Pseudomonas Spp.*(7.8%), *Shigella Spp.* and *Proteus Spp.*(0.8% each). Within Gram Positive Organism all isolates were found to be Cocci. Among Gram Positives isolates *S.aureus* was the predominant (73.9%), followed by *S.saprophyticus* (21.8%), *S. pneumonia* (2.4%) and finally *E. faecalis* (1.6%).The Gram Positive and Gram Negative isolates with count and percentage are shown in Table 2.

Our study showed that the organisms isolated were resistant to one or more drugs of different class.The resistance percentage of the Gram Positive isolates and Gram Negative isolates are shown in Table 3 and Table 4 respectively.

Table 2. Gram Positive and Gram Negative isolates with count and percentage

Gram Negative Organisms			Gram Positive Organisms		
Organism	Count	Percentage (%)	Organisms	Count	Percentage (%)
E. coli	46	35.9	Enterococcus faecalis	2	1.7
Klebsiella Spp.	51	39.8	S. aureus	88	73.9
Proteus Spp.	1	0.8	S. saprophyticus	26	21.8
Pseudomonas Spp.	10	7.8	Streptococcus pneumoniae	3	2.5
ShigellaSpp.	1	0.8	**Gram Positive Total**	**119**	**100.0**
Gram Negative Total	**128**	**100.0**			

Gram Positive organisms showed resistance to many antibiotics but Chloramphenicol, Norfloxacin, Novobiosin showed excellent efficacy with no resistance at all.

Staphylococcus aureus was found resistant to most of the antibiotics. Highest resistance was found against Extended Spectrum Penicillin; Amoxicillin (82.5%) followed by antistaphylococcal, Oxacillin (64.7%) which also indicated the high percentage of MRSA isolates in our study and similarly high resistance was found against Cotrimoxazole (58.7%). Low resistance was found against Tetracycline (8.5%), Amino glycosides; Amikacin and Gentamycin (6.3% and 12.5% respectively), Carbapenem; Imepenem (20%) and Cloxacillin (11%) and hence these drugs were found to be effective.

S. saprophyticus showed highest resistance against Oxacillin (90%), followed by Amoxicillin (85.7%), similarly high resistance was found against Macrolides; Erythromycin and Azithromycin (85.7% and 75% respectively). Low resistance was found against Aminoglycosides and Tetracycline groups.

Streptococcus pneumoniae isolates showed highest/complete resistance against Macrolides, Sulfomethoxazole trimethoprim and Glycopeptide. Fluoroquinolone, Ciprofloxacin was found comparatively effective with less resistance (33.3%).

Enterococcus Spp. isolates were comparatively sensitive to common antibiotics.

Table 3. Gram Positive Organism Antibiotic Resistance

Gram Positive Organism Antibiotic Resistance				
Antibiotics	*Enterococcus Spp.*	*S. aureus*	*S. saprophyticus*	*Streptococcus pneumoniae*
Amikacin	0.0%	6.3%	8.0%	0.0%
Gentamicin	0.0%	12.5%	0.0%	0.0%
Ceftriaxone	0.0%	18.8%	52.0%	50.0%
Caftazidime	0.0%	0.0%	0.0%	0.0%
Cephotaxime	0.0%	0.0%	0.0%	0.0%
Ofloxacin	0.0%	50.0%	37.5%	0.0%
Ciprofloxacin	0.0%	39.4%	45.0%	33.3%
Norfloxacin	0.0%	0.0%	0.0%	0.0%
Erythromycin	50.0%	49.1%	87.5%	100.0%
Azithromycin	0.0%	48.1%	75.0%	100.0%
Amoxicillin	0.0%	82.5%	85.7%	50.0%
Piperacillin/Tazobactam	0.0%	50.0%	0.0%	0.0%
Chloramphenicol	0.0%	0.0%	0.0%	0.0%
Cotrimoxazole	50.0%	58.7%	70.0%	100.0%
Tetracycline	0.0%	8.5%	4.0%	0.0%
Cloxacillin	0.0%	11.3%	60.0%	50.0%
Oxacillin	0.0%	64.7%	90.0%	0.0%
Vancomycin	0.0%	23.1%	33.3%	100.0%
Linezoline	0.0%	50.0%	33.3%	0.0%
Novobiosin	0.0%	0.0%	0.0%	0.0%
Imipenim	0.0%	20.0%	0.0%	0.0%

Table 4. Gram Negative Organism Antibiotic Resistance

Antibiotics	Acinetobacter-Spp.	E. coli	Klebsiel-laSpp.	Proteus Spp.	Pseudomonas Spp.	Shigel-laSpp.
Amikacin	50.0%	13.6%	38.3%	0.0%	0.0%	0.0%
Gentamycin	66.7%	0.0%	25.0%	0.0%	0.0%	0.0%
Tobramycin	100.0%	0.0%	100.0%	0.0%	0.0%	0.0%
Ceftriaxone	72.2%	67.4%	71.4%	0.0%	30.0%	0.0%
Ceftazidime	100.0%	100.0%	100.0%	0.0%	100.0%	0.0%
Cephotaxim	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Nalidixicacid	100.0%	50.0%	0.0%	0.0%	0.0%	0.0%
Ofloxacine	100.0%	20.0%	58.3%	0.0%	0.0%	0.0%
Ciprofloxacin	58.8%	72.5%	61.5%	0.0%	25.0%	0.0%
Norfloxacin	0.0%	100.0%	33.3%	0.0%	0.0%	0.0%
Erythromycin	75.0%	92.0%	93.5%	100.0%	100.0%	0.0%
Azithromycin	100.0%	17.6%	33.3%	0.0%	0.0%	0.0%
Amoxicillin	90.0%	96.9%	100.0%	0.0%	100.0%	100.0%

PiperacillinTazo-bactam	33.3%	0.0%	33.3%	0.0%	100.0%	0.0%
Chloramphenicol	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Cotrimoxazole	87.5%	66.7%	68.4%	0.0%	88.9%	0.0%
Nitrofurantoin	100.0%	0.0%	50.0%	0.0%	0.0%	0.0%
Tetracycline	25.0%	38.1%	25.5%	0.0%	87.5%	0.0%
Novobiosin	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Imepenem	28.6%	0.0%	6.2%	0.0%	33.3%	0.0%
Cefepime	0.0%	100.0%	100.0%	0.0%	0.0%	0.0%

Within Gram Negative organisms isolated, we found most of the organism to be MDR except Shigella which was only resistant to Amoxicillin (100%) and Proteus which was only resistant to Erythromycin (100%). We found high level resistance by all isolates to Ceftazidime (100%), Cefepime (100%).

In our study *Klebsiella Spp.* was found to be highly resistant to 3rd generation Cephalosporin; Ceftriaxone (71.4%) and Ceftazidime (100%) but sensitive to Cefotaxime. Fourth generation Cephalosporins; Cefepime was found to be completely resistant (100%). Among Macrolides it was found to be less resistant to Azithromycin (33.3%) as compared to Erythromycin (93.5%). Among Quinolones, it was found completely sensitive to NA, Norfloxacin was less resistant (33.3%) than Ofloxacine (58.3%) and Ciprofloxacin (61.5%). High resistance was also found against Cotrimoxazole (68.4%). Amoxicillin was 100% resistant and Piperacillin/Tazobactam showed low resistance (33.3%). Low resistance were found against Aminoglycosides; Gentamycin (25%) and Amikacin (38.3%) while Tobramycin was resistant. Carbapenem was highly active against *Klebsiella Spp.* with very low resistance against Imepenem (6.2%). Chloramphenicol was also completely sensitive.

E. coli was found to be highly resistant to 3rd generation Cephalosporin; Ceftriaxone (67.4%) and Ceftazidime (100%). Fourth Generation Cephalosporin; Cefepime was also 100% resistant. Among Macrolides high resistance found against Erythromycin (92%) while Azithromycin was very less resistant (17.6%) in comparison. Among Quinolones, Ofloxacine was less resistant (20%) than Ciprofloxacin (72%) and Norfloxacin (100%). Cotrimoxazole was found to be highly resistant (66.7%). High resistance was found against Amoxicillin (96.9%) while Piperacillin/ Tazobactamwere not at all resistant. Aminoglycosides were found to be very effective with low resistance against Amikacin (13.6%) and no resistance against Gentamicin and Tobramycin. Tetracycline was also less resistant (38.1%). Carbapenem was found to fully sensitive.

Acinetobacter Spp. was found highly resistant to 3rd generation Cephalosporin; Ceftriaxone (72.2%), Cefotaxime and Ceftazidime (100% both). High resistance was also found against Macrolides; Erythromycin (75%) and Azithromycin (100%). Among Quinolones Nalidixic Acid and Ofloxacine were completely resistant (100%) and Ciprofloxacin was also highly resistant (58.8%) while no resistance was found against Norfloxacin. Cotrimoxazole was found to be highly resistant (87.5%). Extended spectrum Penicillin drug Amoxicillin was highly resistant (90%) but Piperacillin/Tazobactam was less resistant (33.3%). Aminoglycosides were also relatively ineffective with high resistance against Gentamicin (66.7%) and Tobramycin (100%) but Amikacin was 50% sensitive. Tetracycline was less resistant (25%) and similarly less resistance was found against Carbapenem antibiotic Imepenem (28.6%).

Pseudomonas Spp. showed low resistance to 3rd generation Cephalosporin; Ceftriaxone (30%) but complete resistance to Ceftazidime. Among Macrolides; Erythromycin was fully resistant (100%) while Azithromycin was fully sensitive. Fluoroquinolone drug, Ciprofloxacin was less resistant (25%) while Ofloxacine was fully sensitive. High resistance against Sulphomethoxazole trimethoprim (Cotrimoxazole) was found (88.9%). Extended spectrum penicillin antibiotics Amoxicillin and Piperacillin Tazobactam both were fully resistant. Aminoglycosides were found to be fully sensitive and so was Chloramphenicol with all 100% sensitivity rate. Tetracycline was highly resistant (87.5%). Carbapenem (Imepenem) was also effective with less resistance (33.3%).

The prevalence of multidrug resistance was found to be high among *E. coli* (78.26%), *Klebsiella Spp.* (70.58%), *Pseudomonas Spp.* (70%) and highest among *Acinetobacter Spp.* isolates (89.47%).

Antibiotic resistance by all organisms is shown in Table 5. Full resistance was seen against Ceftazidime and Cefepime while no resistance was observed against Chloramphenicol and Novobiosin.

Table 5: Overall antibiotic resistance

Antibiotics	Resistance
Amikacin	16.8%
Gentamicin	21.1%
Tobramycin	42.9%
Tetracycline	20.7%
Ceftriaxone	47.2%
Cephotaxim	50.0%
Ceftazidime	100.0%
Ciprofloxacin	51.3%
Ofloxacine	49.0%
Norfloxacin	33.3%
Nalidixicacid	30.0%
Chloramphenicol	0.0%
Erythromycin	74.7%
Azithromycin	42.1%
Nitrofurantoin	57.1%
Amoxicillin	90.2%
Cotrimoxazole	67.4%
Piperacillin/Tazobactam	31.2%
Imepenem	11.1%
Cefepime	100.0%
Linezoline	50.0%
Vancomycin	27.6%
Cloxacillin	26.9%
Oxacillin	74.1%
Novobiosin	0.0%

Discussion

Pyogenic infections require the laboratory identification and confirmation along with the antibiotic susceptibility test reports for the proper management of these infections. Nowadays it has been observed that the pyogenic pathogens are gradually showing a high degree of antibiotic resistance. So there must be clear knowledge about the pattern and antimicrobial susceptibility to choose the correct treatment regimen.

In this study gram negative isolates were slightly higher than gram positive similar to study done by *Kala Yadhav M L and Ashmitha Raja* and study done by Muluye[1,15].

In our study S. aureus was the most common organism isolated among all Gram Positive isolates and among Gram Negative isolates *Klebsiella Spp.* was most prevalent followed by *E. coli* opposite to many studies[1,16,17,18].

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a global phenomenon with a prevalence rate ranging from 2% in the Netherlands and Switzerland, to 70% in Japan and Hong Kong[20,21] whereas a prevalence rate of 26.12 % (Total sample) and 28.73% (from Pus sample) was reported in Nepal[22]. Our study, however, showed very high prevalence of MRSA i.e. 64.7% of *S. aureus* isolates.When Vancomycin is considered for treatment, choice inevitably requires the need for in vitro susceptibility testing of every isolate of MRSA in the clinical laboratories owing to emergence of Vancomycin resistant *Staphylococcus aureus* (VRSA) in various parts of world[22]. The Percentage of VRSA was 23.1%.

With regard to CoNS, high rates of Oxacillin resistance (more than 70%) were found worldwide while in our study S. saprophyticus, a common isolate 90% resistance to oxacillin[20].

Streptococcus Spp. showed high resistance to Sulfomethoxazole trimethoprim (Cotrimoxazole), Macrolides followed by extended spectrum penicillin (Amoxicillin) and Fluoroquinolone comparable to study conducted in Kathmandu[23]. In the same study Cephalosporin had shown higher efficacy despite of increasing resistance but in our study these didn't prove much effective. 33.33% isolates of *Streptococcus Spp.* were found to be MDR in our study.

Resistance of *Klebsiella Spp.* was found to be higher against Ciprofloxacin (61.5%) as in a study conducted in National Medical College and Teaching Hospital, Nepal [24]. *Klebsiella species* and *E. coli* were sensitive to Aminoglycosides, Quinolones and Piperacillin/ Tazobactam showed high resistance to Cefepime and Cotrimoxazole similar to, and high resistance was found against 3rd generation Cephalosporin, Cefotaxime which is even higher than the study conducted in Chalmedaanandrao Institute Of Medical Sciences, Karimnagar, Andhrapradesh, India[25]. Low sensitivity of *E. coli* to Ceftriaxone and Cotrimoxazole

and higher sensitivity to Aminoglycoside, Carbapenem and Piperacillin/Tazobactam was similar to the APUA newsletter[26] except Imepenem and Piperacillin/ Tazobactam both showed full efficacy against *E. coli* isolate and in contrast Gentamicin showed no resistance in our study.

Our study found 78.26% *E. coli* was MDR which was slightly higher than those reported previously in Nepal[27], and 70.58% *Klebsiella Spp.*was found to be MDR and this percentage is lower than the same reported in the similar study.

Acinetobacter Spp. showed high resistance against most of the commonly used antibiotics of different classes with most of the isolates i.e. 89.47% being MDR similar to the study conducted at National Institute of Neurological and Allied Sciences, Nepal[28]. In our study this is the highest percentage of MDR isolates among all.The rate of MDR *Acinetobacter Spp. in our study* is higher than reported in past study[27].

Pseudomonas Spp. showed least resistance to Ciprofloxacin (25%) among Fluoroquinolone similar to study conducted by Van Eldre J[29].

According to number, organisms were found to be highly resistant to antibiotics of Cephalosporin group, Macrolides, Extended spectrum penicillin (Amoxicillin), followed by Cotrimoxazole, Fluoroquinolone, and Oxacillin. Other antibiotics were relatively less resistant with percentage lower than 50. Carbapenem, Amino glycosides, Tetracycline, Fluoroquinolone, Quinolonesetc showed potent efficacy. No resistance was found against Chloramphenicol and Novobiosin in our study. Among antistaphylococcal antibiotics Vancomycin and Cloxacillin seem to be effective. The important finding in our study also includes the high resistance of the Gram Negative bacilli to the 3rd generation Cephalosporin; Ceftriaxone, Cefotaxime and Ceftazidime.

CONCLUSION

Out of 847 samples received, we found growth in 247 samples (29.17 %). Among these 247 positive samples, 128 (51.8%) were identified as Gram Negative Organism and 119 (48.2%) were Gram Positive Organism. Commonest gram negative isolate was *Klebsiella Spp.* (39.8%) and *S.aureus* was the most predominant (73.9%) among Gram Positive Organism. The highest of number of growth was seen in Pus (59.5%) followed by Sputum (21.1%) and Aspirate (6.5%).

Our study showed that the organisms isolated were resistant to one or more drugs of different class. The most sensitive drug for gram positive were Chloramphenicol, Cephotaxim and Norfloxacine. The only drug which was 100% sensitive to Gram Negative organism was Chloramphenicol.

REFERENCES

1. *Dagnachew M, Yitayih W, Getachew F, Tesfaye N, Kasaw A, Belete B, Habtie T and Feleke M. Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/or wound discharge at Gondar university hospital. BMC Research Notes. 2014;7:619. http://dx.doi.org/10.1186/1756-0500-7-619*
2. *Howard RJ, Ravitch MM, Steichen FM. Host against Infections. Current Problems in Surgery. New Eng, J Med. 1980;12:1823–1830.*
3. *Singh S, Khare M, Patidar R.K., Bagde S, Sahare K.N., Dwivedi D, Singh V. Antibacterial Activities Against Pyogenic Pathogens. Int J Pharm Sci Res. 2013;4(8):2974-2979. http://dx.doi.org/10.13040/IJPSR.0975-8232.4(8).2974-79*
4. *Androulla E: Outbreaks of human infection caused by pyogenic streptococci of Lancefield groups C and G. Journal of Medical Microbiology. 1989;29:207-219. http://dx.doi.org/10.1099/00222615-29-3-207*
5. *Thomas KH. Surgical Wound Infection, an Overview. Am J Med. 1981;70:712–718. http://dx.doi.org/10.1016/0002-9343(81)90602-1*
6. *Collier M. Recognition and management of wound infections Wounds.2004 [cited 2014 May 15]. Available from:http://www.worldwidewounds.com/2004/january/Collier/ Management-of- Wound-infections.html*
7. *Cornaglia G, Hryniewicz W, Jarlier V, Kahlmeter G, Mittermayer H, Stratchounsk Li and Baquero F. European recommendations for antimicrobial resistance surveillance. Clinical microbiology and infection. 2004;10(4):349-383. http://dx.doi.org/10.1111/j.1198-743X.2004.00887.x*
8. *Elmer WK, Stephen DA, William MJ, Schreckenberger PC, Winn WC. Colour Atlas And Textbook of Diagnostic Microbiology. 5th ed. Philadelphia: Raven Publisher; 1997. 69–120*
9. *Saravanan R, Raveendaran V. Antimicrobial resistance pattern in a tertiary care hospital: An observational study. J Basic ClinPharma. 2013;4(3):56. http://dx.doi.org/10.4103/0976-0105.118797*

10. Okonko IO, Soleye FA, Amusan TA, Ogun AA, Ogunnusi TA, Ejembi J. Incidence of Multi-Drug Resistance (MDR) Organismsin Abeokuta, Southwestern Nigeria. Global J. Pharmacology. 2009;3(2):69-80.

11. CDC. Antimicrobial Susceptibilities Among Group B Streptococcus Isolates, Active Bacterial Core Surveillance (ABCs) (2010). http://www.cdc.gov/abcs/reports-findings/ survreports/gbs10-suscept.html [Accessed 7/23/2013].

12. El-Astal Z. Bacterial pathogens and their antimicrobial susceptibility in Gaza Strip, Palestine. Pakistan J. Med. 2004;20(4):365-70.

13. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. In: Collee JG, Duguid JP, Fraser AG, Marmion BP, eds. Mackie and McCartney Practical Medical Microbiology. 14th ed. New York: USA: Churchill Livingstone; 1996. 131-149.

14. National committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests NCCLS document M2-A7, Approved Standard, 7th edition: Wayne, PA: NCCLS, 2000.

15. ML KY, Raja A. Bacteriological profile and antibiogram of the gram negative clinical isolates from a tertiary care centre. Int J Res Health Sci [Internet]. 2014 Jul 31;2(3):734-9

16. Rao DR, Basu R, Biswas DR. Aerobic Bacterial Profile and Antimicrobial Susceptibility Pattern of Pus Isolates in a South Indian Tertiary Care Hospital. Surgery. 2014;36:35.29.

17. Neelima PKD, Suresh P. Bacteriological profile of wound infection in rural hospital in RR district. International Journal of Medical Research & Health Sciences. 2013; 2(3): 469-73. http://dx.doi.org/10.5958/j.2319-5886.2.3.081

18. Shrestha B, Basnet RB. Wound infection and antibiotics sensitivity pattern of of bacterial isolation PMJN. 2009;9(1):1-5

19. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug resistant, extensively drug resistant and pandrug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012;18(3):268-281. http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x

20. Diekema D, Pfaller M, Schmitz F, Smayevsky J, Bell J, Jones R, et al. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clinical Infectious Diseases. 2001;32(2):S114-S32. http://dx.doi.org/10.1086/320184

21. Saikia L, Nath R, Choudhury B, Sarkar M. Prevalence and antimicrobial susceptibility pattern of methicillin–resistant Staphylococcus aureus in Assam. Indian J Crit Care Med. 2009;13:156-8.http://dx.doi.org/10.4103/0972-5229.58542

22. Pandey S., Raza MS., Bhatta CP. Prevalence and Antibiotic Sensitivity Pattern of Methicillin- Resistant Staphylococcus aureus in Kathmandu Medical College Teaching Hospital. Journal of Institute of Medicine. 2012;34(1):13-17.

23. Shakya G, Adhikari BR. Ten-years surveillance of antimicrobial resistance pattern of Streptococcus pneumoniaein Nepal. African Journal of Microbiology Research. 2012;6(20):4233-38.

24. Upadhayay AK, Parajuli P. Extended spectrum β-lacctamase producing multidrug-resistant Klebsiella species isolated at National Medical College and Teaching Hospital, Nepal. Asian Journal of Pharmaceutical and Clinical Research. 2013;6(4):161-4.

25. Anjum P, Rajkumari VA, Pavani N, Yamini K, Sabarinathan T. Antibiotic Susceptibility Pattern of Pyogenic Bacterial Isolates in Sputum. IOSR Journal of Pharmacy and Biological Sciences.2013;7(2):13-7. http://dx.doi.org/10.9790/3008-0721317

26. APUA-Nepal Newsletter. Comparative Study Of Sensitivity Patterns Of Common Isolates In Tribhuvan University Teaching Hospital (TUTH) 2013;10(1).

27. Panta K, Ghimire P, Rai SK, Mukhiya RK, Singh RN, Rai G. Antibiogram Typing of Gram Negative Isolates In Different Clinical Samples Of A Tertiary Hospital. Asian Journal of Pharmaceutical and Clinical Research. 2013;6(1):154-6.

28. Khanal S, Joshi DR, Bhatta DR, Devkota U, Pokhrel BM. β-lactamase-producing multidrug-resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at National Institute of Neurological and Allied Sciences, Nepal. ISRN microbiology. 2013. http://dx.doi.org/10.1155/2013/847569

29. Van Eldere J. Multicentre surveillance of Pseudomonas aeruginosa susceptibility patterns in nosocomial infections. Journal of Antimicrobial Chemotherapy. 2003;51(2):347-52. http://dx.doi.org/10.1093/jac/dkg102