Proceedings of First International Conference on Infectious Diseases and Nanomedicine-2012 (ICIDN-2012)

December 15-18, 2012, Kathmandu, Nepal

November 2013

EDITORIAL BOARD

The **"Proceedings of First International Conference on Infectious Diseases and Nanomedicine-2012 (ICIDN-2012)"** is published and distributed by**:**

ICIDN-2012 Secretariat

Post Box No. 24411, Kathmandu, Nepal

Website: www.icidn2012.com, E-mail: icidn2012@gmail.com,

EDITORIAL

It is our pleasure to release the "Proceedings of *First International Conference on Infectious Diseases & Nanomedicine-2012 (ICIDN-2012)",* held in Kathmandu, Nepal from December 15-18, 2012. The ICIDN-2012 organized by Nepalese Association of Medical Microbiology (NAMM) and Nepal Polymer Institute (NPI) in association with Kathmandu University (KU), Kavre was attended by scientists and delegates from 21 countries throughout the globe.

The success of the conference and subsequent agreement for the publication of selected papers of high scientific values in Springer Book Series- *Advances in Experimental Medicine and Biology* in two volumes further encouraged us to publish this proceeding with selected papers presented in the conference. The Proceedings of ICIDN-2012 which contains full articles related to research presented during the ICIDN-2012 is envisioned as compilations of the updated information on the status of infectious diseases and nanomedicine in Nepal and will provide an important reference material to the readers.

Each manuscript is meticulously reviewed by the editors based on the comments from the reviewers. Every efforts has been taken to minimize the error in production. However, the opinion, expressed by the authors in their papers is not the official views of the organizer.

We are thankful to all the reviewers for their time and comments/suggestions. Thanks also go to the advisors of the editorial board, contributors of this volume and all the participants of the conference as well as the well wishers.

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Proceedings of First International Conference on Infectious Diseases & Nanomedicine-2012 (December 15-18, 2012, Kathmandu, Nepal)

Screening for Silver Nanoparticles Biosynthesizing Capacities of Different Medicinal Plants

Bimala Subba [1] , Ram Chandra Kandel [2], Bibek G.C. [2], Khem Raj Giri [2], Depen Khatri [2], Pramod Aryal [1], Werner Lebek [3], Reinhold Godehardt [3] and Rameshwar Adhikari [1,*]

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ABSTRACT

In present study, biosynthesis of silver nanoparticles (AgNPs) was studied by using extract of leaves of plants belonging to five different families. The nanoparticles were examined by using UV-visible spectroscopy and transmission electron microscopy (TEM). The first results suggest that out of five plants, the leaves extract of A. vulgaris is exceptionally advantageous for the bio-reduction of silver ion leading to the synthesis of AgNPs. In this paper, we report on the synthetic procedure, spectroscopic and microscopic analyses and antimicrobial activity of AgNPs synthesized by using the leaves extracts of selected plants having traditional medicinal values. More systematic studies are needed for unfolding the great potential of Nepalese herbs in nanoparticles research.

***Keywords**: Silver nanoparticles (AgNPs), medicinal plants, biosynthesis, antibacterial properties*

INTRODUCTION

Today, noble metal nanoparticles are intensely studied because of their extensive application to new technologies in chemistry, electronics, medicine, and biotechnology [1-3]. Silver is also important in this respect. Silver nanoparticles (AgNPs) have many applications; for example, they might be used as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors, as catalysts in chemical reactions, for biolabelling, and as antimicrobials [4-6]. The use of silver nanoparticles as antibacterial agent is relatively new. Because of their high reactivity due to the large surface to volume ratio, nanoparticles play a crucial role in inhibiting bacterial growth in aqueous and solid media. Silver containing materials can be employed to eliminate microorganisms on textile fabrics [7,8] or they can be used for water treatment [9]. Although many techniques of synthesizing AgNPs, such as physical, chemical and biological methods have been reported in the past few years, among them biological methods has gained more importance as other methods are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks [10,11]. In biological methods, nanoparticles are synthesized by using microorganisms [12,13] and plants or their extracts.

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction of metal salts. In other words, plant mediated synthesis of nanoparticles could be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures [14]. Many reports have been published in the literature on the biogenesis of AgNPs using several plants extract, such as Neem leaves [15], natural rubber leaf [16], Aloe vera plant extracts [17], starch [18], bamboo leaves [19], switchgrass [20] etc. Basically, the biosynthesizing ability of the plants depend on the nature of different water soluble plant metabolites such as alkaloids, phenolic compounds, terpenoids as well as different co-enzymes. The potential of different plant extracts for biosynthesis of metal nanoparticles has been recently reviewed [2]. However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants [21]. The biogenesis of AgNPs using plant extracts from Nepal has not been investigated so far. In this study, the biogenesis of AgNPs using five medicinal plants of Nepal has been investigated for the first time. (Table 1). We also studied the antimicrobial activity of silver nanoparticles prepared from aqueous extract of *A. vulgaris* against some Gram negative bacteria.

EXPERIMENTAL PART

Chemicals

All analytical chemicals such silver nitrate, ammonia solution were purchased from Merck chemicals, India and the microbial media (such as nutrient agar) and its components such as ampicillin) were purchased from Hi-Media, Mumbai, India. All the aqueous solutions were prepared using three fold distilled water.

Plant Materials

Fresh leaves of *J. adhatoda* and *A. vulgaris* were collected from Pashupatinath temple area; the leaves of *P. guajava*, *Azadirachta indic*a were from Jorpati area in Kathmandu. The leaves of *Aloe vera barbadensis* were collected from Bagdol area of Lalitpur, Nepal during the month of July, 2012. The plants were hence authenticated by Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Table 1*: Selected plant taxa with related information*

Scientific Name	**Local name**	**Family**	**Some traditional medicinal uses of the plants**
Justicia adhatoda	Asuro	Acanthaceae	1. To treat bleeding gums, piles and peptic ulcers as well as internal hemmorhage 2. Expectorant, antispasmodic and good blood purifier; to reduce fever 4. To speed up the child birth
Aloe vera Barbadensis	Ghee Kumari	Cactus	1. Treatment of burn injuries, skin scarring, and also for healing of cuts, wounds and scratches 2. Treatment of skin diseases such as Eczema, dry skin diseases, inflammations, and allergies as well as insect bites, stings. 3. Treatment of pains associated with Arthritis and other rheumatic pains 4. Treatment of piles and haemorrhoids, ulcers, scores etc.
Artemisia vulgaris L.	Titepati	Asteraceae	1. Anti-inflammatory, antispasmodic, carminative and anthelmintic properties 2. Treatment of dysmenorrhoea 3. In the induction of labour or miscarriage
Psidium guajava L	Amba	Myrtaceae	1. Treatment of gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums etc.
Azadirachta indica	*Neem*	Meliaceae	1. Uses in tiredness, cough, fever, loss of appetite, worm infestation, excessive thirst, and diabetes 2. Anti-leprotic anti-hemorrhoids and anthelmintic medicament, used against neuromuscular pains, and against insect poisons

Microorganisms

The assessment of antibacterial activity was carried out using two Gram negative strains (*Escherichia coli* and *Salmonella typhi*). Both bacterial strains were identified and obtained from Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Synthesis of Ag nanoparticles

20 g each of fresh leaves of each collected plant were weighed, thoroughly washed with sterile distilled water and then ground well using mortar and pestle. The ground material was mixed with 100 mL of sterile distilled water and then transferred in 500 mL Erlenmeyer flasks. The content of

each flask was boiled for 3 minutes, and filtered using cheese cloth filter. The filtrates were stored in sterile beakers maintained at 4 °C for further use. The same procedure was used for the plant extracts preparation from the leaves of all the plants in question. For the synthesis of AgNPs, 2.5 mL of 25% ammonia solution was added to 5 mL of 0.001 M $AgNO_3$ solution followed by addition of 5 mL of the leaves extract. The observation of faint yellow color after 24 hours of reaction indicated the formation of silver nanoparticles.

Antibacterial activity study

Approximately 20 mL of molten and cooled nutrient agar media was poured into sterilized Petri dishes. The plates were left overnight at room temperature to allow any contamination to appear. The bacterial test organisms were grown in nutrient broth for 24 hours. A 100 mL nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Sterile discs of 5 mm diameter were impregnated with 50 µl of AgNPs synthesized from an aqueous leaf extract of *A. vulgaris*. Plant extract was used as negative control and dishes of ampicillin was used as a positive control. The plates were then incubated at 37 °C, and examined for evidence of inhibition zones, which appear as a clear area around the discs. The diameter of such zones was measured manually using a millimeter scale ruler. The mean diameter of inhibition zone for each organism was determined from triplicate measurements and the diameter was expressed in millimeters [22].

Nanoparticles characterization

The colloidal solution of silver nanoparticles (AgNPs) obtained was purified by repeated centrifugation at 12,000 rpm for 20 min. The resulting solution was then diluted with a small aliquot of 100 µL of sample with 1 mL of distilled water and assayed with ultra-violet (UV)-visible spectrometry using a Perkin Elmer UV-visible absorption spectrophotometer.
Specimens for transmission electron microscopy (TEM) were prepared by dropping the solution onto the carbon coated copper grids and allowing the solvent to evaporate. The TEM investigations were performed on a Leo 912 transmission electron microscope (TEM) operated at an accelerating voltage of 120 kV.

RESULTS AND DISCUSSION

Biosynthesis of silver nanoparticles by the aqueous extract of *A. barbadensis, J. adhatoda, A. vulgaris, P. guajava and A. indica* were investigated. Biosynthesis of AgNPs by each plant extracts was confirmed by color change from clear to pale yellow brown after 24 h of reaction period. Fig. 1 shows the photographs of sample solutions containing silver nitrate (left test tube) and silver nitrate in the presence of extract of *A. vulgaris* after completion of the reaction (right test tube). The appearance of a yellowish-brown color (right test tube) confirms the existence of colloidal AgNPs in the solution.

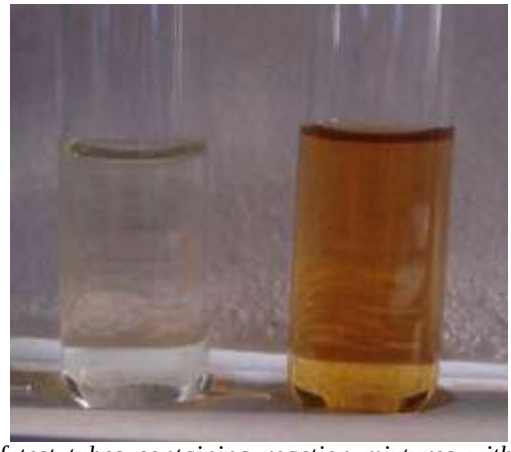

Figure 1*. Photographs of test tubes containing reaction mixtures without (left) and with (right) extract of A. vulgaris after 24 hours*

The formation of silver nanoparticles is more precisely confirmed by UV-visible absorbance spectroscopy, one of the most widely used techniques for signatory characteriztion of nanoparticles [23]. The results are presented in Fig. 2.

The absorption spectrum of yellowish-brown silver nanoparticle solution prepared with the extract of *A. vulgaris* showed a surface plasmon resonance band (longitudinal vibration) at 300 nm, Fig. 2. The intensity of peak changes with the experimental conditions but the peak position remains the same. The observation is slightly different from some of the literature works [19,20] which have reported the wavelength of surface plasmon resonance at higher values.

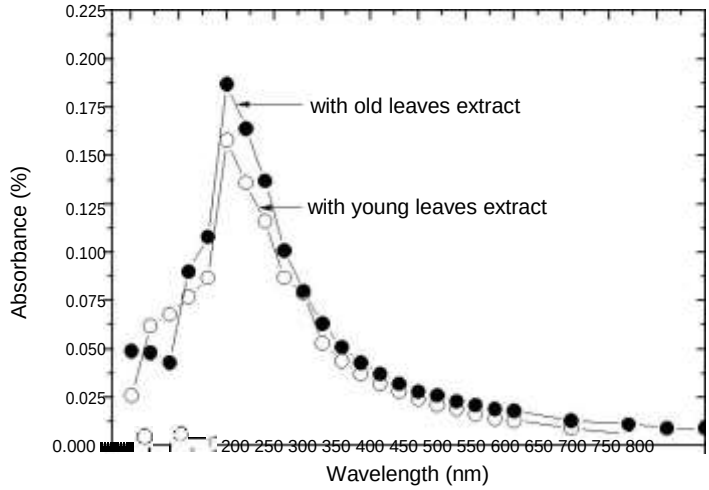

Figure 2. *UV-visible spectrum of AgNPs colloidal solution prepared using A. vulgaris leaves extracts*

The absorption spectrum of yellowish -brown AgNPs solution prepared by other plant extracts did not show any surface plasmon absorption band indicating the aggregation of the nanoparticles (results not discussed here).

AgNPs synthesized using *A. vulgaris, J. adhatoda, P. guajavaleaf, A. barbadensis* and *A. indica* extracts were subjected to TEM investigations. It was found that the formation of silver nanoparticles or aggregates has occurred with all plants extracts. We present the TEM results obtained for the AgNPs prepared with *A. vulgaris* and *J. adhatoda* only. Spherical shaped AgNPs with diameter ranging from 5 nm to 40 nm were observed for the mixture containing leaves of extracts of *A. vulgaris* (Fig. 3a). In agreement with the spectroscopic results, there is, however, a wide disparity in the size of the nanoparticles. In other case, aggregation of the nanoparticles was observed which was responsible for absence of clearly defined surface plasmon resonance bands in the UV-visible spectra which can be correlated with the presence of much larger aggregates which are several hundred nanometers in diameter (Fig. 3b). The aggregation behaviour might have been originated due to lack of suitable capping agent.

Although the biosynthesis of silver nanoparticle by using extract of *A. barbadensis and A. indica* has been reported in the literature [15,17], the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants [21]. Thus, the samples used in this study and those in the previous studies might be representing different chemotypes. Indeed the mechanism of biosynthesis of metal nanoparticles is complex and is under debate. The underlying mechanism of biosynthesis of nanoparticles has been recently studied by Kesharwani and co-workers [3].

Among the screened five plants, *A. vulgaris* was found to be the best candidate for the biosynthesis of silver nanoparticles. Thus, in this case, the effect of plant age on the synthesis of the silver nanoparticles was further studied by carrying out the reaction using young and old leaves. The results revealed that old leaves were found to be more effective in the reducing the silver ions to AgNPs which may be attributed to the presence of larger amount of photochemical in the older leaves.

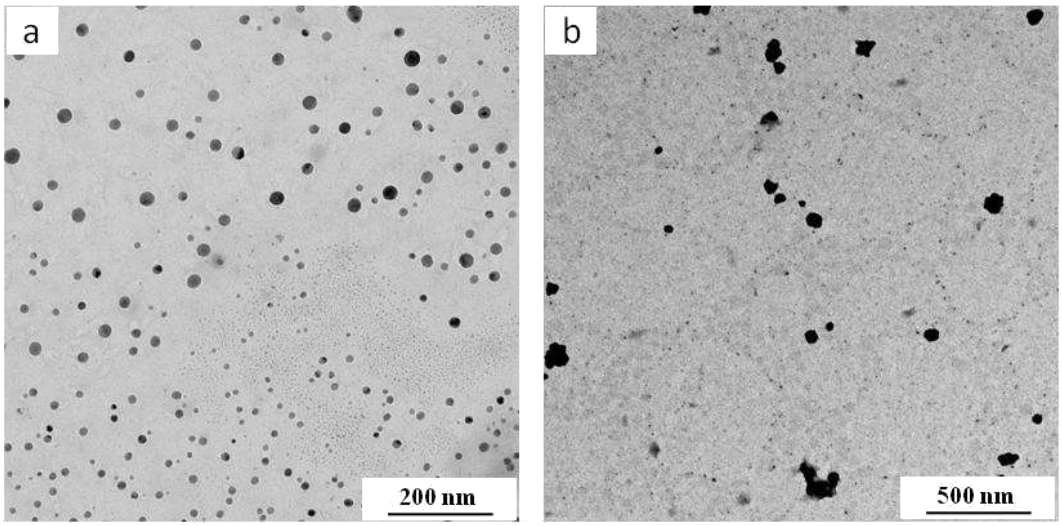

Figure 3. *TEM micrographs of AgNPs prepared using different plant extracts: a) A. vulgaris and b) J. adhatoda*

Finally, the antibiotic activity of silver nanoparticles (*A. vulgaris*) was investigated against two pathogenic organisms such as *E. coli* and *S. typhi*. The results are indexed in Table 2. The AgNPs were effective against both strains of microorganisms. The diameter of zone of inhibition was 7 mm for *E. coli* and 8 mm for *S. typhi*.

Table 2: *Zone of inhibition by AgNPs prepared using A. vulgaris leaves extract*

Specimen used	D (mm) for *E. coli*	D (mm) for *S. typhi*
Standard (5 µg/dish)	14	10
AgNPs (50µL)	7	8

D = diameter of inhibition zone

CONCLUSIONS

We initiated exploring the potential of different Nepalese herbs for the biosynthesis of silver nanoparticles (AgNPs) by allowing the silver ions to interact with different plants' leaves extract. The AgNPs thus produced were characterized by UV-visible spectroscopy and transmission electron microscopy (TEM). The primary screening has demonstrated that *A. vulgaris* has remarkable potential of biosynthesizing uniformly shaped, but rather polydisperse silver nanoparticles (AgNPs) having significant antibacterial efficiency. Some other plant extracts produced no favorable results as the particles were assembled into large agglomerations several hundred nanometers in diameter. The biosynthetic route provided rather simple, straight forward one-pot method for the preparation of metal nanoparticles.

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Multidrug Resistant Bacterial Pathogens among Patients Visiting a Tertiary Care Referral Hospital in Central Nepal

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ABSTRACT

Antibiotic resistance is a global problem to both the community and hospital settings. Imprudent use of antimicrobial agents is the commonest predisposing factor for the development of resistance. The present study has been carried out with a view to find out the rate of multidrug resistant (MDR) bacterial isolates and their antibiotic susceptibility pattern in different clinical specimens of patient visiting B and B hospital from June 2011 to March 2012. Samples were processed and bacteria were identified based on the guidelines provided by Clinical and Laboratory Standards Institute. Out of the 940 clinical specimens collected and processed, 460 (48.90%) showed significant growth on culture. Of the total of 12 different bacterial species isolated, gram negative bacteria accounted 77.80% (n=358) while gram positive counterpart were only 22.20% (n=102). Among the gram positive bacteria, S. aureus was found to be the most predominant one (70/102) representing 15.20% of total bacterial isolates. Similarly, Pseudomonas aeruginosa was the dominant gram negative isolate (122/358) representing 26.50% of total bacterial population.

The most effective antibiotics against gram positive bacteria (other than S. aureus) was chloramphenicol with sensitivity of 84.40%. All the gram positive MDR isolates were 100% sensitive toward antibiotics- cefeperazone, sulbactam and vancomycin. Out of the total 70 S. aureus isolates, 17.10% (12) were found to be methicillin resistant. All MRSA isolates were sensitive to vancomycin and only 75.00% of MRSA were sensitive to chloramphenicol while most of the MSSA isolates were sensitive to the antibiotics tested.
On the other hand, the most effective antibiotics against gram negative bacteria was amikacin and chloramphenicol with sensitivities of 79.10% and 59.80% respectively. Gram negative isolates showed very high resistance pattern towards amoxicillin (86.03%) followed by ceftriaxone (61.45%), and ciprofloxacin (58.38%). In total, 65.40 % of isolates of gram negative bacteria was found to be MDR. Among different species, 94.10% of Acinetobacter baumanii, 68.80% of Klebsiella spp., 65.20% of Enterobacter spp., 61.50% of P. aeruginosa, and 60.00% of Escherichia coli were MDR gram negative bacteria. Amikacin, meropenem and imepenem was found to be sensitive to most of the MDR GN isolates. This study reveals the high rate of multidrug resistant gram negative bacteria, hence cautions must be exercised whenever antibiotics therapy to be administered. Moreover, the results of this study could be of help to the decision makers in order to implement prevention programs so as to prevent the emergence and spread of antimicrobial resistance.

***Keywords**: Bacteria, antibiotic susceptibility pattern, MDR, MRSA, Nepal*

INTRODUCTION

Resistance towards antibiotics is a global problem in the hospitals as well as in the community and is listed at the top of Center for Disease Control (CDC)'s list of emerging infectious threats to the public health. Primary mode of transmission of antimicrobial resistance in a hospital is patient to patient spread via hands and equipments of health care workers. Problems faced are especially with methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE) and multidrug resistant gram-negative bacilli (MDR-GNB) like *Klebsiella pneumoniae, Enterobacter* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. [1-3].

Resistance of gram-negative bacteria is consequently emerging in the hospital setting, the resistance is mainly due to the rapid increase of extended-spectrum β-lactamases (ESBLs) in *K. pneumoniae, E. coli* and *Proteus mirabilis*; high level third-generation cephalosporin β-lactamase resistance among *Enterobacter* spp. and *Citrobacter* spp. and multi drug resistant (MDR) in *P.aeruginosa, Acinetobacter* spp. and *Stenotrophomonas maltophilia* [4]. The

emergence of antimicrobial resistance pathogens now threats the discovery of potent antimicrobial agents. Antimicrobial resistance has resulted in increased morbidity and mortality as well as health care costs [5].
The rate of infections caused by MDR organism is relatively higher in the developing country like Nepal. This possesses a serious public health threat. In Nepal, the resistant pathogens are more common because of the inappropriate use of antibiotics and most often due to a failure to finish the full course of treatment. In both the circumstances, improper dose of antibiotic not only fails to completely eliminate the organism from diseased individual but also encourage further growth of the most resistant strains [6].

Present study has been designed to provide supportive information on the occurrence, distribution and the incidence of bacterial infection and their antibiotic susceptibility pattern in one of the tertiary care hospitals of central Nepal. The findings of rate of MDR isolates and new epidemic strains of bacteria will be of help for rapid and effective infection control policy.

EXPERIMENTAL PART

Material and methods

The study was carried out from June 2011 to March 2012 in the Microbiology Department of B and B hospital, Gwarko, Lalitpur, Kathmandu, Nepal. A total of 940 clinical specimens (including wound swab, pus swab, different tips like foley'scatheter tips and CVP tip different tubes like tracheostomy tube, tracheal suction tube, and different body fluids like pleural fluid, ascitic fluid and cerebrospinal fluid) collected from patients following case definition of suspected infection were included in the study. A descriptive cross sectional type of study was done. Various demographic information of the patients were recorded by completing the questionnaires, with informed consent. All specimens were transported to the laboratory as soon as possible and processed for microbiological analysis using standard protocol. The sample was inoculated on Blood agar (BA), Mac-Conkey Agar (MA) and Chocolate Agar (CA) plates. MA plates were incubated aerobically at 37ºC for 24 to 48 hours while BA plates and CA plates were incubated in microaerophilic condition at 37ºC for 24 to 48 hours [7].
The standard microbiological technique, which involved colony morphology, staining reaction, biochemical properties was followed for the identification of organisms [8]. Gram positive cocci were identified following catalase, O/F, coagulase, bacitracin and optochin sensitivity test. For Gram negative organisms, biochemical tests (Oxidase, Catalase, Methyl red, VogesProskaeur test, Citrate utilization test, Indole production test, Triple sugar iron agar test, SIM test and Urease test) were performed by inoculating a single isolated colony from media on to the respective biochemical media. Haemolysis in blood agar for gram positive bacteria, morphological and cultural characteristic on Mac-Conkey agar was observed especially for gram negative bacteria. After 18 to 24 hours of incubation, standardized inocula of bacteria obtained from well isolated, morphologically similar colonies were tested against antibiotics by modified Kirby Bauer disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) [9]. *S. aureus* was screened for methicillin resistant by modified Kirby Bauer disc diffusion method using Cefoxitin disc (30 µg) as per standard guidelines provided [9]. Gram negative bacteria such as *E.coli, Pseudomonas* spp., *Acinetobacter* spp., *Klebsiella* spp. were defined as multidrug resistant if they were resistant to one or more classes of antibiotics [10]. Data were analysed by SPSS v. 20 software.

RESULTS AND DISCUSSION

Distribution of bacterial growth in different clinical samples

Among the 940 clinical samples processed, 351 (37.30%) were pus swab, 234 (24.90%) were wound swab, 237 (25.20%) were different tips, 69 (7.30%) were different body fluids and 49 (5.20%) different tubes. Of all the samples analyzed, significant growth of pathogens were observed in 460 (48.90%) samples. Further, growth positivity was maximum in different tubes (63.30%) followed by different tips (62.00%), wound swab (48.71%) and pus swab (45.90%). Lowest percent of growth was recorded in body fluids (10.10%). The high rate of growth positivity highlights the need for maintenance of proper sanitation in the hospital as these isolates are source of major nosocomial outbreaks in the hospital environment [11]. Various investigators elsewhere in the world have reported higher growth positive cases which was in agreement with the findings of our present study [12,13] . Several investigators have argued that long stay in hospitals, the use of invasive instruments and invasive procedures might increase the higher growth rate, which make patient more prone to infection Panlilio *et al.* [14].

Higher number of wound specimens in this study might be due to the higher number of surgical wound infections and its substandard hygienic condition. Similar results were obtained by Khan *et al*.[15] According to them, out of 550 clinical specimens from hospitalized patients; more than 83.00% of samples were from wound swabs and 53.30%. Dufour *et al.* [16] reported low growth positivity among the body fluid samples and different tips samples which was similar to the present findings. The reason for the low prevalence from body fluids, catheter tips, CVP tips and different tubes might be due to less sample size included [16]. Okonko *et al.* [17] reported that delay in the transportation of specimens to the laboratory may adversely affect the growth of fastidious organism which could result in low growth rate. In addition, prior use of antibiotic might also limits the growth of such pathogens.

Growth among outdoor and indoor patients

Of all the samples analyzed, 635(67.6%) samples were from indoor patients and 304 (32.3%) samples were from outdoor patients. Growth was observed in 53.90% and 38.40% cases of indoor and outdoor patient respectively. The high rate of growth positive was found to be in indoor patients than outdoor patient, which was similar to the study of Taiwo *et al.*[18]. 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 agent [19].

Types of bacterial isolates

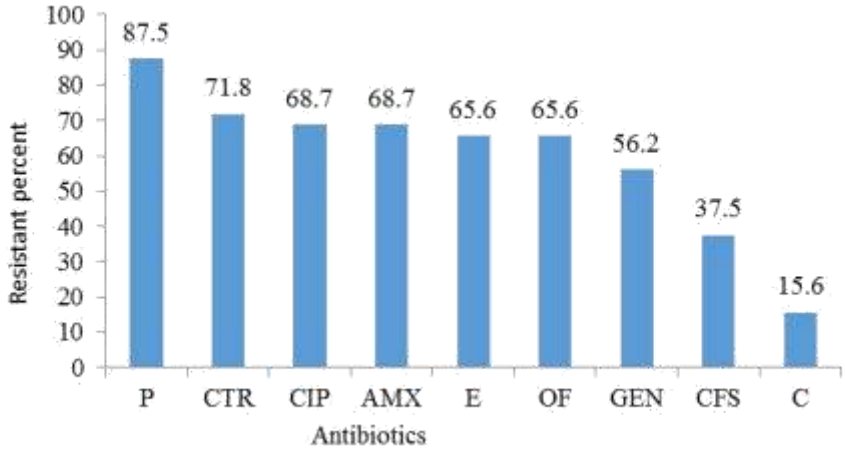
Out of 460 bacterial isolates, only 22.20% (n=102) were gram positive while 77.80% (n=358) 102 were gram negative. Among gram positive isolates, *S. aureus* was the most common (15.20%) and β-haemolytic streptococci was the least (0.2%) (see Table 1). In gram negative side, *P. aureginosa* was the dominant isolate (26.50%) followed by *E. coli* (17.40%), *Acinetobacter* spp. (11.40%), *Klebsiella* spp. (10.40 %), *Enterobacter* spp. (10.00%), *Citrobacter* spp. (1.70%) and *Proteus* spp. (0.70%) to the least (see Table 1).

Table 1: *Pattern of bacterial growth*

Organism Isolated	Total	Percent
Gram Positive Bacteria		
β haemolytic streptococci	1	0.20
Non haemolytic streptococci	5	1.10
Enterococcus spp.	16	3.50
CONS	10	2.20
S. aureus	70	15.20
Gram Negative Bacteria		
Proteus spp.	3	0.70
Citrobacter spp.	8	1.70
Enterobacter pp.	46	10.00
Klebsiella spp.	48	10.40
A. baumanii	51	11.10
E.coli	80	17.40
P. aeruginosa	122	26.50
Total	**460**	**100**

Antibiotic susceptibility pattern of gram positive bacteria other than *Staphylococcus aureus*

All the tested isolates (n=32) demonstrated highest level of resistant towards penicillin (87.50%) followed by others (See Figure 1). The most effective antibiotic was chloramphenicol with sensitivity of 84.40%. All the MDR isolates were 100% sensitive towards antibiotics formulation: cefeperazone, sulbactam and vancomycin used to detect gram positive MDR.



P= Penicillin; CTR= Ceftriaxzone; CIP= Ciprofloxacin; AMX= Amoxicillin; E= Erythromycin; OF= Ofloxacin; GEN= Gentamycin; CFS= Cefoperazone/sulbactam; C= Chloramphenicol

Figure 1. *Antibiotic susceptibility pattern of gram positive isolates other than S.aureus*

These findings are in accordance with a study conducted in Nepal by Shrestha [20].

Antibiotic susceptibility pattern of *S. aureus*

Of all the 70 isolates of *S. aureus* tested, MRSA was reported in 17.10% (n=12) cases. All the MRSA isolates were resistant to penicillin and cloxacilin whereas 98.30 % of MSSA were resistant to penicillin and 53.40% towards amoxycillin. MSSA isolates were sensitive to most of the other antibiotics tested (see Table 2). All MRSA isolates were sensitive to vancomycin while only 75% of MRSA were sensitive to chloramphenicol.

Table 2: *Antibiotic susceptibility pattern of S. aureus*

Table 2: *Antibiotic susceptibility*	**MSSA(n=58)**	*pattern of S. aureus*	**MRSA (n=12)**
Antibiotics tested	**Resistant**	**%**	**Resistant %**
Penicillin	57	98.30	12	100.00
Cloxacillin	2	3.40	12	100.00
Erythromycin	16	27.60	11	91.70
Amoxycillin	31	53.40	9	75.00
Ciprofloxaxin	13	22.40	8	66.70
Ofloxacin	10	17.20	7	58.30
Ceftriaxone	2	3.40	7	58.30
Gentamycin	5	8.60	6	50.00
Chloramphenicol	1	1.70	3	25.00

MRSA= methicillin resistant S. aureus, MSSA= methicillin sensitive S. aureus

Studies on MRSA have shown a wide variation. This study is similar to the study carried by Weigelt *et al*.[21] Kownhar *et al*.[22] , however the study of Taiwo *et al*.[18] showed 29.00%; Chen and Zhang [23] showed 82.50% MRSA growth which is higher than this study. The study conducted by Naik and Deshpande[24] showed 8.00% of MRSA which is in contrast to this study. Mumtaz *et al*.[25] found *S. aureus* to be highly sensitive to ofloxacin (82.00%) followed by ciprofloxacin (67.00%) and erythromycin (62.00%), which was similar to the present study.

Antibiotic susceptibility pattern of gram negative bacteria

Among the total gram negative isolates (n=358), a very high percentage of resistance was toward amoxicillin (86.03%) followed by ceftriaxone (61.45%) and ciprofloxacin (58.38%). The most effective antibiotics were found to be amikacin and chloramphenicol with sensitivities of 79.10% and 59.80% respectively. In total, 65.40 % of isolates of gram negative bacteria was found to be MDR. Among different species, *94.10% of Acinetobacter baumanii, 68.80% of Klebsiella spp., 65.20% of Enterobacter spp., 61.50% of P. aeruginosa, and 60.00% of Escherichia coli were MDR gram negative bacteria.* For MDR organism (n=234), sensitivity test of following antibiotics- cefeperazone/ sulbactam, piperacillin/tazobactam, meropenem and imepenem was performed; meropenem and imepenem was found to be sensitive to most of the isolates.

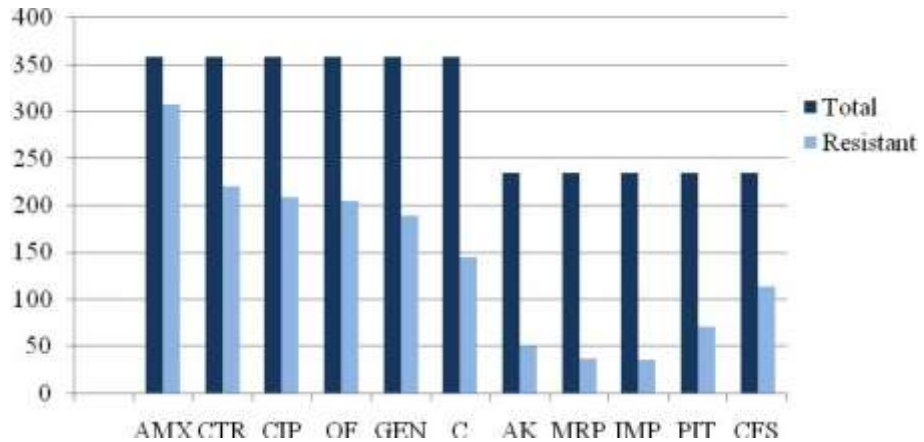


Figure 2. *Antibiotic susceptibility pattern of gram negative bacteria*

Our findings showed relatively higher resistance pattern of gram negative bacteria against amoxicillin and ceftriaxone than those studies conducted by Kumari [26] as well as Tuladhar [27]. This may be due to the hydrolysis of beta lactam ring of the β lactam antibiotics by the action of beta lactamase enzymes or due to the loss of outer membrane protein and production of class Amp C β lactamases and altered target proteins [28]. Ciprofloxacin and ofloxacin resistant was also higher than the study carried by Ahemed *et al.*[29] but lower as compared to Javiya *et al.*[30]. This development of resistance to quinolones is due to the decrease in binding of the target quinolones to the enzymes because of changes in DNA gyrase enzyme/ topoisomerase enzyme.[30] Present study indicates that majority of the gram negative isolates were more sensitive to imipenem and amikacin as compared to the other antibiotics tested which was in accordance to the study conducted by Ahemed *et al.* [29]and therefore these may be considered the drugs of choice for the treatment of nosocomial infections at tertiary care hospitals.

In the study carried out by Ahemed *et al.,*[29] of the 148 clinical isolates, 50% of MDR *P. aeruginosa* strains were commonly isolated from pus sample which was in accordance to the present study. However, in a study carried out by Baweja,[31] out of total 12,107 samples received during six months, 2987 bacteria were grown (24.70%), of which 904 (30.30%) were multidrug resistant bacteria which was slightly lower than present study. The increase in number of immune compromised hosts, debilitating effects of prolonged hospitalization, application of medical equipments (airways, catheters etc) and improper infection control measures has contributed to the dissemination of MDR-bacteria in hospital settings [32-34].

Antibiotic resistant pattern of gram negative bacteria to first and second line antibiotics

Among 122 isolates of *P. aeruginosa*, (83.60%) were resistant to amoxicillin followed by, chloramphenicol (54.10%), ceftriaxone and ciprofloxacin (50.00%), ofloxacin and gentamycin (49.20%) Among 80 isolates of *E. coli*, (78.80%) were resistant to amoxicillin. 35.00% and 27.50% of isolates were resistant to chloramphenicol and gentamicin respectively. *A. baumanii* were resistant to most of the antibiotics. Only 11.80% of isolates were resistant to chloramphenicol.

Among the second line antibiotic used, amikacin, imepenem and meropenem was found to be the most effective antibiotics among MDR gram negative bacteria.

Table 3: *Antibiotic resistant pattern of gram negative bacteria to first line and second line antibiotics*

First line Antibiotics used	**CIP** N %	**OF** N %	**AMX** N %	**C** N %	**CF** N %	**GEN** N %
P. aeruginosa (n=122)	61 (50.00)	60 (49.20)	102 (83.60)	66 (54.10)	62 (50.8)	60 (49.20)
E. coli (n=80)	44 (55.00)	45 (56.30)	63 (78.80)	22 (27.50)	48 (60.0)	28 (35.00)
A. baumanii (n=51)	47 (92.20)	47 (92.20)	47 (92.20)	6 (11.80)	47 (92.2)	49 (96.10)
Enterobacter spp.(n=46)	22 (47.80)	20 (43.40)	45 (97.80)	24 (52.10)	27 (58.7)	25 (54.35)
Klebsiella spp.(n=48)	28 (58.30)	27 (56.30)	42 (87.50)	23 (47.90)	29 (60.4)	21 (43.80)
Citrobacter spp.(n=8)	4 (50.00)	4 (50.00)	7 (87.50)	0 (0.00)	0 (0.00)	0 (0.00)
Proteus spp. (n=3)	2 (66.60)	2 (66.60)	2 (66.60)	0 (0.00)	0 (0.00)	0 (0.00)

Second line Antibiotics used	**AK** N %	**MRP** N %	**IMP** N %	**PIT** N %	**CS** N %
P. aeruginosa (n=75)	12(16.0)	15(28)	11(14.6)	23 (30.6)	37 (49.3)
E. coli (n=48)	9 (18.7)	5 (10.4)	2(4.1)	9 (18.7)	33 (68.7)
A. baumanii (n=48)	15 (31.4)	6 (12.5)	16(33.4)	20 (41.2)	24 (50.0)
Enterobacter spp. (n=30)	8(26.7)	2 (6.7)	2(6.7)	10 (33.4)	6 (20.0)
Klebsiella spp. (n=33)	5(15.2)	8 (24.2)	4(12.1)	9 (27.3)	14(30.3)

*(*Figure in the parenthesis is in percentage. N= number of resistant isolates)*

CONCLUSION

Among the gram negative isolates, *P. aeruginosa* was found to be most predominant and was resistant to amoxycillin, ciprofloxacin and ceftriazone. Hence, based on the observation of present study we recommend use of amikacin, carbapenems and piperacillin/tazobactam to control the infection by *P. aeruginosa.* Vancomycin and chloramphenicol and may present a unique opportunity in the management of staphylococcal infections. Similarly, amikacin and carbapenems are the most effective drugs for the MDR gram negative bacteria. The study reveals the high rate of MDR GNB (65.4%), hence cautions must be exercised whenever antibiotics therapy to be administered. Regular antimicrobial susceptibility surveillance is essential for regional monitoring of resistance pattern and to develop methods to formulate a potent and effective antibiotic policy. Therefore, it is an important issue which has to be addressed by the policy makers in order to formulate strict antibiotic prescription policy in our country.

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Antibiotic Susceptibility Pattern of Bacterial Isolates from Wound of Patients visiting Kanti Childrens' Hospital, Kathmandu

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ABSTRACT

Among all pediatric injuries, infection of wound is the most damaging and may cause lifelong disfigurement, dysfunction, and even death. A cross sectional study was conducted from November 2011 to July 2012 at Kanti Childrens' Hospital, Kathmandu, Nepal to isolate bacteria responsible for wound infection among children attending the hospital and to assess their antibiotic susceptibility pattern. Standard microbial techniques were employed to isolate and identify the bacteria and antimicrobial susceptibility testing of the isolates was performed by Kirby Disc diffusion method Out of the 420 pus samples processed, 47% (197) were growth positive. Among the growth positive samples, 63% (128) were gram negative and 37% (75) were gram positive bacteria belonging to thirteen different genera. Staphylococcus aureus (32%) was found to be the most predominant bacterial isolate followed by Pseudomonas aeruginosa (23.6%), Acinetobacter spp (14.8%), E. coli (13.8%), Klebsiella spp (5.9%), Coagulase negative Staphylococci (3.4%), Citrobacter freundii (1.5%), Proteus vulgaris (1.5%), Enterobacter spp (0.9%), Streptococcus pyogenes (0.9%), Citrobacter koseri (0.9%), Serratia marcescens (0.9%) and Proteus mirabilis (0.4%). Additionally, the rate of infection was higher in surgical wounds followed by burn wounds. The most effective antibiotic against gram positive bacteria was found to be tobramycin followed by cephalexin while amoxycillin and cotrimoxazole were in the least effective line. Similarly, imipinem, tobramycin and piperacillin were the most effective drugs against gram negative bacterial isolates. Amoxycillin, ciprofloxacin, cephalexin and ceftazidime were least effective.

Keywords: *Pus samples, wound infection, gram positive & gram negative bacteria, antibiotic susceptibility*

INTRODUCTION

Wound, a disruption in the normal tissue resulting in a variety of cellular and molecular sequelae leads to exposure of subcutaneous tissues and provides a moist and nutritional environment supporting microbial colonization and proliferation [1]. The presence of microorganism on wound lead to four outcome- contamination, colonization, critical colonization and infection [9]. Wound infection can be monomicrobial or polymicrobial [5]. During initial stage, few organisms are present on wound, which may or may not colonise. When those contaminants overcome host immune system, it leads to infection. The organisms multiply causing harm to the host. Normal flora or harmless organisms might not cause deleterious effect to the host. However, the virulence factor of the organism and the host immune status determine whether it is harmful infection or harmless colonisation. The potential pathogens commonly encountered in wound infections are *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes,* Coagulase negative Staphylococci, *Pseudomonas* spp., *Acinetobacter* spp., *Escherichia coli, Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp. and anaerobes like *Peptostreptococcus* spp, *Clostridium* spp [3].

Wound contaminants are likely to originate from three different sources: the environment (exogenous microorganisms in the air or those introduced by tramautic injury), the surrounding skin (skin normal flora) and endogenous sources involving mucous membranes [3]. However, any wound to be infected depends upon – type, depth and location of wound, immune status of host, virulence of organism and other predisposing factors such as age, underlying illness and nutritional status [2].

Wound infection cause greater fear both in developed and developing countries. Young children are more susceptible to infection because of the presence of low level of antibodies until they develop sufficient IgG antibody levels at age of 10 years [4]. In the event of infection, a wound fails to heal, the patient suffers increased trauma, treatment costs rise, and general wound management practices become more resource demanding [6].

Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world with increasing drug resistant strains [7]. This might be reflection of inappropriate use of the existing antibiotics due to unavailability of guideline regarding selection of drugs [8]. To help physician's choice of appropriate antibiotics, up-to-date information on local prevailing strains and their drug sensitivity pattern is very crucial to treat patients. Although study on wound infection has been frequently done in adults, few studies have been carried out among children population. Previous studies regarding burn infection among pediatric have been done but scanty information are available regarding wound infection in pediatric. Therefore this study is conducted to determine the bacterial etiologies of wound infection and their antimicrobial susceptibility pattern among the pediatric patients attending Kanti Childrens' Hospital, a national tertiary care referral center based at Kathmandu. This study thus aimed to provide a baseline to select appropriate antibiotics for the treatment of wound infection and helps in minimizing the alarmingly increased trend of antibiotic resistance.

EXPERIMENTAL PART

Sample collection and transport

Pus samples were collected from pediatric patients attending Kanti Childrens' Hospital, from November 2011 to July 2012, using either sterile cotton swabs or stoppered syringes by medical officer. Aseptic techniques were employed during collection in order to avoid contamination from commensals and external sources. These samples were then transported to the Microbiology Laboratory for processing as early as possible to avoid desiccation and to prevent the growth of unwanted microbes.
Ethical approval was obtained from the hospital authority and informed consent was taken from each patients and their parents. A total of 420 pus samples were collected from different types of wounds including burn wound, surgical wound and others such as cut, abrasions, bite wound.

Sample processing

Samples obtained in laboratory were processed and analyzed following standard criteria [7]. Gram staining was performed from the smears made from pus. The samples were separately inoculated onto different sets of Blood agar (BA) and Mac Conkey agar (MA) media. Blood agar plates were incubated at 37˚C for 24 in candle jar and Mac Conkey agar plate plates at 37˚C for 24 hours.

Isolation and identification of the isolates

After incubation, the both plates were observed for the growth of bacteria and colony morphology was noted. Gram staining was also performed from the isolated colony using standard protocol. The morphological and gram character of the bacteria was noted. Catalase and oxidase tests were performed. Furthermore, gram negative isolates were inoculated into various biochemical media namely: TSI, SIM, Citrate agar and urease agar. The organisms were identified based on the biochemical properties observed after 24 hours of incubation at 37˚C [7].

Antibiotic susceptibility test

Antibiotic susceptibility testing was done by Kirby Bauer Disk diffusion method. After identification of the organisms, 0.5 Mac Farland equivalent of suspension of the organism was prepared. A sterile cotton swab was used and the excess suspension was removed by gently pressing and rotating the swab to the inside wall surface of the tube. The prepared suspension was then swabbed evenly on MHA plate and BA plate. MHA was used for all gram positive and gram negative isolates except *Streptococcus* spp. Blood agar was used for *Streptococcus* spp. Commercially available antibiotic discs were used according to the nature of the organism and zone of inhibition was observed. The result was interpreted by comparing with standard charts[10].

RESULTS AND DISCUSSION

Out of 420 samples from pediatric patient, only 197(47 %) samples showed positive growth. Similar result was obtained in a study conducted by in Kathmandu Model Hospital and in Medicare National Hospital and Research Centre[11, 12].

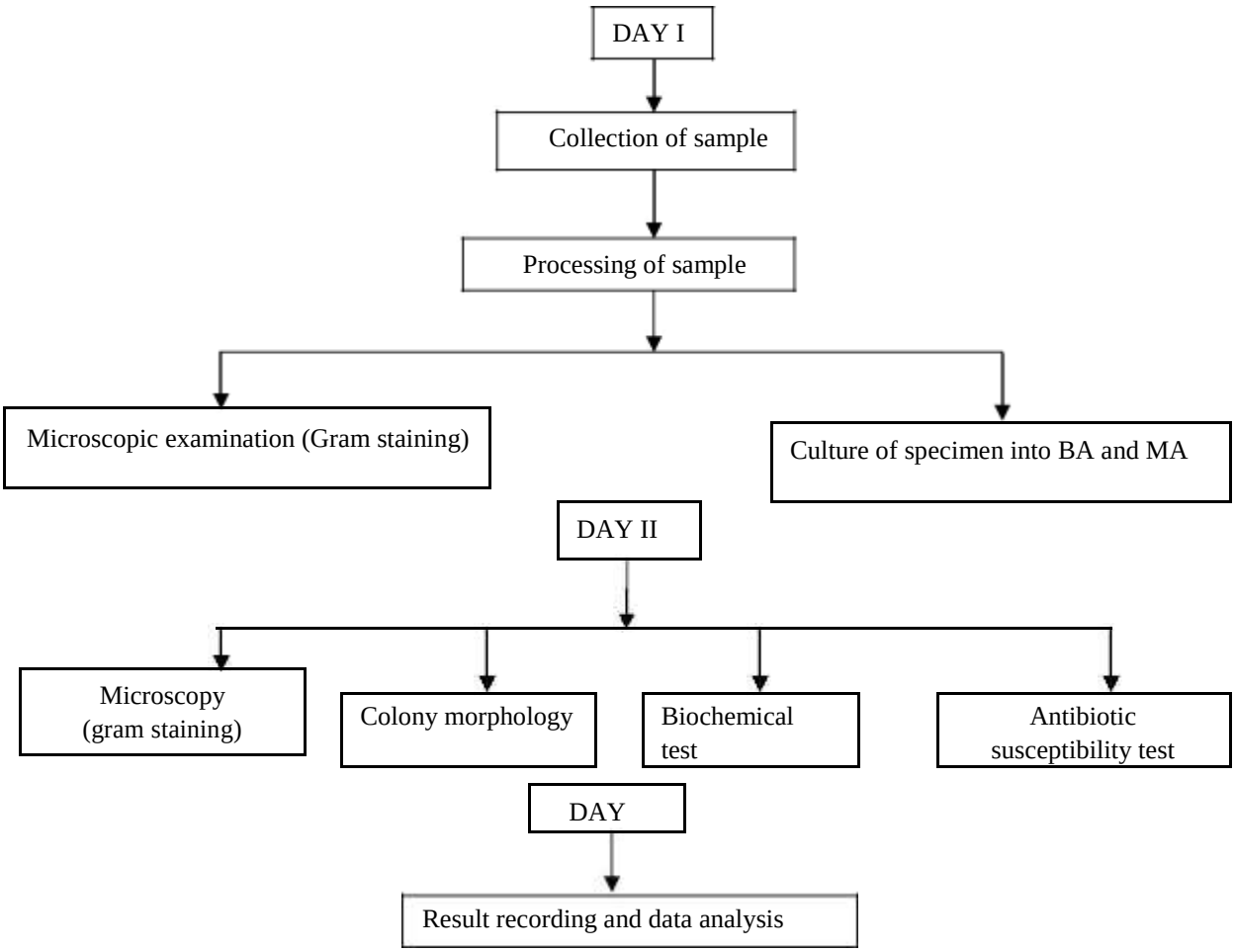

Figure 1. *Flow chart for laboratory analysis of pus sample*

There was significant difference between the rate of infection and type of wound ($p < 0.05$) (see Table 1). Surgical wound was found to be the most infected (68.8%) followed by burn wound (50%) and other type of wound (cut, impetigo, erysipelas, accidental wound etc.) (41.6 %) . This result is in contrast to a study conducted among all age patients by Giri *et al* which showed 7.3% for SSI [13]. Similarly 18.7% of SSI was observed in a study conducted by Porras-Hernandez in Mexico [14]. Higher rate of surgical infection in our study might be due to high susceptibility of children towards infection, poor infection control practices, lack of proper supplies for wound care and personal hygiene. These risk factors are reported in most studies of wound infection related to both the adults and pediatrics [15]. Of 195 samples from burn wound, 99 (50%) showed positive growth which is comparable to other studies [16]. This rate of burn wound infection might be because of the fact that burn wound represents a susceptible site for opportunistic colonization by organisms of endogenous and exogenous origin. Also burn unit is important breeding ground for the development and spread of antibiotic resistant bacteria[17].

Table 1*: Growth pattern in different type of wound*

Types of wound	**Positive growth**	**No growth**	**Total**	**p-value**
Burn wound	99(50.8%)	96(49.2%)	195	p<0.05
Surgical wound	11(68.8%)	5(31.2%)	16	
Other types of wound (cut, abrasions)	87(41.6%)	122(58.4%)	209	
Total	**197**	**224**	**420**	

Of 197 positive samples, 203 bacteria were isolated including polymicrobial among which 128 (63%) were gram negative bacteria and 75(37%) were gram positive. In burn wound gram negative were predominant where as in

surgical wound gram positive organisms were predominant (Figure 2). This is contrary to the study carried out by Bhatta and Lakhey[11]. In burn wound, gram negative (86%) were predominant where as in surgical wound gram positive were predominant (55% and 63% respectively). Isolation of higher number of gram negative organism from burn wound in this study agrees with most of the literatures[13, 18].

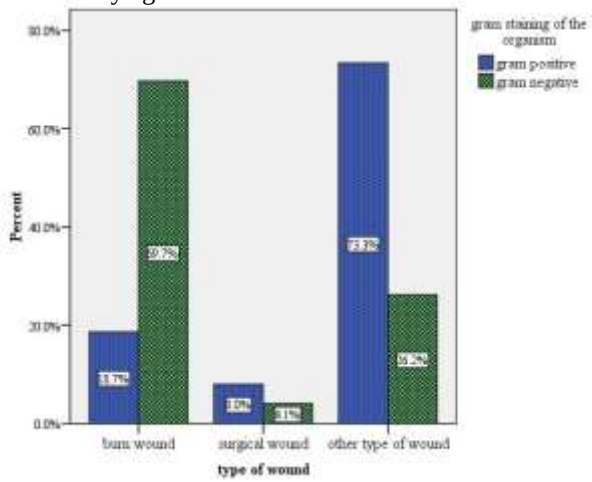

Figure 2. *Pattern of gram positive and gram negative bacteria isolated from different types of wound*

Table 2: *Pattern of microbial isolates in pus collected from different type of wound*

Organism	Burn wound	Surgical wound	Other wound (cut and abrasions)	Total
Staph .aureus	10	6	49	65
Ps. aeruginosa	38	1	9	48
Acinetobacter spp.	26	0	4	30
E. coli	14	4	10	28
Klebsiella spp.	7	0	5	12
CoNS	2	0	5	7
Proteus vulgaris	2	0	1	3
Citrobacter freundii	1	0	2	3
Streptococcus pyogenes	0	0	2	2
Enterobacter spp.	1	0	1	2
Proteus mirabilis	0	0	1	2
Citrobacter koseri	1	0	0	1
Serratia marcescens	0	0	1	1
Total				203

Among the isolates, *Staph. aureus* (32%) was the predominant one followed by *Ps. aeruginosa* (24%), *Acinetobacter* spp (15%) and *E. coli* (14%). Other less common isolates are *Citrobacter* spp, *Proteus* spp., *Enterobacter* spp., *Streptococcus* spp., CONS and *Serratia marcescens*. However second and third most common isolates of our study is in contrast with other studies. In a similar study carried out by Bhatta and Lakhey, Singh *et al* and Shrestha and Basnet, after *Staph. aureus*, *E. coli* was the second predominant isolate[11, 12, 19]. Among 6 samples yielding polymicrobial growth, 4 produced the combination of *E. coli* and *Ps. aeruginosa*. The combination of *Acinetobacter* spp. and *Klebsiella* spp and the combination *E. coli* and *Klebsiell*a spp. was seen in one sample each.

Antibiotic susceptibility pattern of the isolates

Table 3: *Antibiotic susceptibility pattern of Staphylococcus aureus (n=65)*

Antibiotics used	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Amoxicillin	22	33.8	-	-	43	66.2
Tobramycin	45	69.2	14	21.5	5	7.7
Cephalexin	44	67.7	1	1.5	20	30.8
Ciprofloxacin	38	58.5	3	4.6	24	36.9
Co- trimoxazole	28	43.1	-	-	37	56.9
Cefoxitin	50	76.9	8	12.3	7	10.8
Erythromycin	41	63.1	5	7.7	19	29.2

The isolated *Staph. aureus* were mostly susceptible to cefoxitin. 76.9% of the total *Staph. aureus* isolates were susceptible to cefoxitin. Tobramycin was the second drug most effective against the *Staph. aureus* isolates (69.2%) followed by cephalexin (67.7%). Similarly, 58.5% of the isolates were susceptible to ciprofloxacin and 43.1% of the isolates were susceptible to co-trimoxazole. The least effective drug was found to be amoxicillin (33.8%). Similar result was obtained in a study conducted in Tribhuvan University Teaching Hospital (TUTH), Kathmandu Model Hospital and Medicare National Hospital and Research Centre [11,12,19]. However in a study conducted in India by Rajput *et al*, Staphylococci isolated from burn wound was highly resistant to erythromycin (84%) and co-trimoxazole (80%)[18]. In this study, 10.8 % of Staphylococci isolates were resistant to cefoxitin. *Staph aureus* resistant to cefoxitin is considered as Methicillin Resistant *Staph. aureus*. MRSA has emerged as a major pathogen worldwide. The reported prevalence of MRSA in Nepal shows an increasing trend; 29.1% in 1990 and 61.6% in 2003 [20, 21].

All the isolates of *Ps. aeruginosa* in this study were susceptible to imipenem except one isolate. 86% of the *Ps. aeruginosa* isolates were susceptible to piperacillin. Tobramycin, amikacin and ciprofloxacin was effective against 39.6%, 27.1% and 25% of isolates respectively. Ceftazidime was found to be least effective against *Ps. aeruginosa.* This result agrees with other study [18, 22]. However this result was contrary to a study carried out in Kathmandu Model Hospital in which *Pseudomonas* isolates from burn wound showed high sensitivity to ceftazidime, ciprofloxacin, amikacin and tobramycin[12]. In US more than 90% of the isolates were susceptible to amikacin, 80-90% of isolates were susceptible to ceftazidime while 70-80% of isolates were susceptible to ciprofloxacin[23].

Table 4: *Antibiotic susceptibility pattern of Ps. aeruginosa (n=48)*

Antibiotics used	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Ceftazidime	7	14.6	2	4.2	39	81.2
Amikacin	13	27.1	6	12.5	29	60.4
Piperacillin	41	86	-	-	5	10.4
Ciprofloxacin	12	25	4	8.3	32	66.7
Tobramycin	19	39.6	6	12.5	23	47.9
Imipenem	47	97.9	1	2.1	-	-

Table 5: *Antibiotic susceptibility pattern of Acinetobacter spp. (n=30)*

Antibiotics used	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Ceftazidime	2	6.7	-	-	28	93.3
Amikacin	8	26.7	6	20	16	53.3
Piperacillin	13	43.3	10	33.3	6	20
Ciprofloxacin	1	3.3	-	-	29	96.7
Tobramycin	17	56.7	9	30	4	13.3
Imipenem	26	86.7	2	6.7	2	6.7

Imipinem was most effective against *Acinetobacter* spp. 56.7% of the *Acinetobacter* spp isolates were susceptible to tobramycin. Piperacillin, amikacin and ceftazidime were effective against 43.3%, 26.7% and 6.7% of isolates respectively. Ciprofloxacin and ceftazidime were found to be least effective against *Acinetobacter* spp. High resistivity of *Acinetobacter* isolates to ceftazidime and ciprofloxacin agrees most of the studies[24, 25, 26].

Tobramycin was the most effective drug against *E. coli* isolates in our study. 71.4% of the isolates were susceptible to tobramycin. Similarly, 60.7% of the isolates showed susceptibility towards co-trimoxazole. However, ciprofloxacin, ceftazidime and cephalexin were effective against 50%, 42.9% and 32.1% of the isolates. The least antibacterial activity against *E. coli* was shown by amoxicillin. Only 21.4% of the *E. coli* isolates were susceptible to amoxicillin. The finding of sensitivity of *E. coli* in this study is analogous to other studies[11, 12, 18, 19].

Table 6: *Antibiotic susceptibility pattern of E. coli (n=28)*

Antibiotics used	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Amoxicillin	6	21.4	-	-	22	78.6
Tobramycin	20	71.4	3	10.7	5	17.9
Cephalexin	9	32.1	1	3.6	15	64.3
Ciprofloxacin	14	50	1	3.6	13	46.6
Co- trimoxazole	17	60.7	-	-	11	39.3
Ceftazidime	12	42.9	-	-	16	57.1

Table 7: *Antibiotic susceptibility pattern of Klebsiella spp. (n=12)*

Antibiotics used	Susceptibility pattern					
	Sensitive		Intermediate		Resistant	
	Number	%	Number	%	Number	%
Amoxicillin	5	41.7	-	-	7	58.3
Tobramycin	9	75	1	8.3	2	16.7
Cephalexin	3	25	-	-	9	75
Ciprofloxacin	6	54.4	-	-	5	45.5
Co-trimoxazole	9	75	-	-	3	25
Ceftazidime	6	50	-	-	6	50

Tobramycin and co-trimoxazole were the most effective drugs against *Klebsiella* spp. ciprofloxacin and ceftazidime were effective against 54.4% and 50% of the isolates. Cephalexin was least effective against *Klebsiella* spp. Similar findings were reported from a study carried out by Rajput *et al* in which 45.5% of isolates were resistant to ciprofloxacin but 80% of isolates were resistant to co-trimoxazole which is in contrast to this study[18]. In a study by Bhatta and Lakhey , *Klebsiella* isolates were 100% sensitive to cephalexin, ofloxacin and ciprofloxacin[11].

All isolates of CoNS were sensitive to cefoxitin. Tobramycin, erythromycin and cephalexin were effective against 85.7% of the isolates. Similarly, 71.4% of the isolates were susceptible to ciprofloxacin and co-

trimoxazole. Amoxicillin was the least effective drug. Tobramycin, ciprofloxacin and ceftazidime were the most effective drugs against *Proteus vulgaris* whereas ciprofloxacin and co-trimoxazole were effective against *Proteus mirabilis*. There were no isolates of *Proteus* spp. having intermediate resistance status. For *Citrobacter freundii*, cefixime was the most effective antibiotic whereas *Citrobacter koseri* was resistant to most of the tested antibiotics (co-trimoxazole, cefixime, ciprofloxacin, amoxicillin). All isolates of *Streptococcus pyogenes* were susceptible to amoxicillin, cephalexin, chloramphenicol, ciprofloxacin and erythromycin. *Serratia marcescens* was susceptible to all tested antibiotics. Sensitivity of *Serratia marcescens* to all antibiotics tested in this study is supported by Giacometti *et al*[26]. *Enterobacter* spp. was resistant to amoxycillin, cephalexin and ciprofloxacin.

As compared to previous study done in the same hospital by Shrestha [27], antibiotic resistivity pattern is increasing. Many factors may have contributed to such level of resistance, including misuse of antibiotics by health professionals and unskilled practitioners. In Nepal, it is a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public thus contributing to the emergence and spread of antimicrobial resistance. Other causal factors can be poor hygienic conditions accounting for the spread of resistant bacteria, and inadequate surveillance, i.e. lack of information from routine surveillance testing of bacterial isolates and surveillance of antibiotic resistance.

CONCLUSION

In our study gram negative bacterial isolates were higher than gram positive bacteria. The most common isolates were *Staph. aureus*, *Ps. aeruginosa* and *Acinetobacter* spp. *Staph. aureus* isolates were resistant to amoxicillin and co-trimoxazole whereas *Ps. aeruginosa* , *Acinetobacter* spp, *E. coli* and *Klebsiella* spp. were resistant to amoxicillin, ciprofloxacin and even resistant to third generation cephalosporin like ceftazidime. These indicate that antibiotic resistance pattern is increasing in alarming trend.

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Leptospirosis: One of the Neglected Infectious Diseases in Nepal

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ABSTRACT

The wide spectrum of clinical symptoms that characterize leptospirosis make its diagnosis to be easily confused with other febrile disease of human and diagnosis is often missed. The present study is done to find leptospirosis association with undiagnosed cases of acute encephalitis syndrome (AES). Between January to December 2008, JE excluded AES serum samples of all age groups and both gender were received at National Public Health Laboratory (NPHL). The serum samples collected from all over the country were tested for anti-leptospiral antibodies (IgM and IgG) with Latex agglutination method (LeptoTek Dri Dot). The results were analyzed using appropriate statistical tools. Out of 778 serum samples were tested for the presence of anti -leprospira antibodies, 51.67 % were found reactive and the reactive cases were higher in age group 6-15 years (146/402) with no more difference by gender. The cases reported were significantly higher during rainy and post rainy season and in ecological distribution more cases were noted in terai and hilly regions compared to mountain. The significant association of leptospirosis with AES cases indicates that leptospirosis may be common in Nepal but the disease has been neglected by not knowing its burden in the country and not having accessible diagnostic tool.

Keywords: *Neglected infectious disease, leptospirosis, febrile illness, serology*

INTRODUCTION

The neglected infections are largely hidden burden of diseases caused by a group of chronic and debilitating parasitic, bacterial and congenital infections, one of which is leptospirosis, a zoonotic bacterial infection [1]. It is an increasingly recognized cause of acute febrile illness through out the tropical and sub tropical regions of the world. Spirochetal infection with *Leptospira* spp. typically occurs when water or soil contaminated with the urine of infected animals comes in contact with human skin or mucus membrane [2-7.] The clinical manifestation of leptospirosis range from mild self limiting febrile illness to severe and potentially fatal illness characterized by Jaundice, renal failure, meningitis, thrombocytopenia and haemorrhage (Weil's disease) . Early in illness, it is often indistinguishable from other common cause of acute febrile illness in tropics such as dengue, malaria, scrub typhus, typhoid and others. For this reason, it is important to distinguish leptospirosis from dengue and viral hemorrhagic fevers, among others, in patients acquiring infections in countries where these diseases are endemic [1, 7]. Leptospirosis has recently caused large outbreaks where the diagnosis was often initially overlooked [8] .

As rapid laboratory confirmation is not available, disease burden in our country is not revealed and it remained undiagnosed and untreated. To know the existence of leptospirosis in Nepal, it is very essential to carry out preliminary study on leptospirosis to make public aware about the disease. In Nepal in response to this need, the present study is conducted to find its association in undiagnosed cases of Acute Encephalitic Syndrome (AES).

MATERIALS AND METHODS

This descriptive cross sectional laboratory based study was conducted at National Public Health Laboratory (NPHL) between January to December 2008. Serum samples of AES cases were collected from all over the country through Government of Nepal and WHO-IPD surveillance network and transported to NPHL by maintaining cold chain. Informed consent was taken before collection of serum samples from AES patients. JE excluded serum samples of AES cases of all age groups and both genders were included in the study. Anti-JE IgM positive, Quantity Not Sufficient (QNS) and CSF samples were excluded from the study. Initially all samples of AES cases were tested for JE by MAC ELISA for the presence of anti-JE IgM antibodies. JE negative serum samples were tested subsequently for anti-leptospiral antibodies (IgM and IgG) by latex agglutination method using Lepto Tek Dri Dot Kit (specificity= 91.0% and sensitivity = 91.2) [9] . The data were analyzed by using appropriate statistical tool (Win-Pepi, Version 1.55 and 1.69; 2003-2007) as statistical package software.

RESULTS AND DISCUSSION

Of the total 1299 serum samples tested for JE infection, 286 (22%) were positive for anti-JE IgM antibodies. Out of 778 undiagnosed AES serum samples processed for the presence of anti-leptospira antibodies, 402 (51.7%) were found reactive (See Table 1).

Table 1: *Demographic profile*

Total serum samples tested for JE:	1299
JE positive:	286 (22%)
Undiagnosed AES serum samples;	1013
Undiagnosed AES srum samples tested for Leptospirosis:	778
Anti-Leptospira antibodies Reactive:	402 (51.7%)
Male	224
Female	178
M: F ratio	1.25:1
Age group of AES cases:	<1 year to >50 years

Observation of 51.7% leptospirosis reactive cases indicates that the large population of the country might be at the risk of leptospirosis. Lack of early and adequate diagnosis of leptospirosis may lead to the maltreatment which may underpin for the severe consequences of the diseases. The different studies also showed that there were cases of leptospirosis throughout the world with the significantly lower positive percentage as compared to the present study. Serological evidence for leptospirosis was found in 17% of subjects in a study in Thai-Myanmar border [10]. Likewise between May and October 2002, a cluster of acute febrile illnesses occurred in the subtropical Andean foothills of Peru. Serologic evidence in villages where disease had been documented showed that the prevalence of IgM antibody to *Leptospira* ranged from 6% to 52% [11]. Approximately 16% of both AFI (141/886) and acute hepatitis (63/392) cases showed seroreactivity to *Leptospira* IgM in a study done in Egypt [12] .

Our study is also comparable with the study done in India where antibodies to leptospires were detected in 322 samples giving an overall seroprevalence of 52.7%. The seroprevalence was highest among agriculture workers (62.5%) followed by sewage workers (39.4%), animal handlers (37.5%), forest workers (27.3%), and butcher (30.0%). Sero-prevalence among control population was 14.7%, which was comparatively less than that of the high -risk population groups [13]. Leptospirosis has been recognized as an important occupational hazard of agriculture manual laborers, sewage workers, animal handlers, forestry workers and other outdoor workers who work in wet conditions, and butchers [6, 14].The present study has limitation in revealing the leptospirosis by occupation which necessitates further study to address the occupational risks and transmission of leptospirosis within the larger population in Nepal.

The reactive cases were higher in age groups 6-15 years (36.3%) followed by 16-30 years (21.4%) and 2-5 years (17.4 %) . Reactivity was least in age group above 50 years with statistically significant difference (see Table 2). There is insignificantly high preponderance of male over female subjects (55.72% over 44.28% respectively) (p = 0.277) which might be the result of higher samples of male patients.

Table 2: *Leptospirosis sero-reactive frequency by age, sex and Ecological distribution*

Sample size	**Total (778)**	**Reactive (402)**	**Reactive % (51.7%)**
Age groups (years)			
<1- 1	96	30	7.50%
1-5	137	70	17.40%
5-15	278	146	36.30%
15-30	146	86	21.40%
30-50	79	48	11.00%
>50	42	22	5.50 %
			P = 0.001
Sex			
Female	330	178	44.28%
Male	448	224	55.72%
			P = 0.277
Ecological distribution			
Terai	368	208	51.7%
Hill	388	183	45.5%
Mountain	22	11	2.8%
			P= 0.036

Except in children below one year of age, the positive (reactive) cases in each age group were above 50% of the total tested. Leptospirosis is more prone to the older age group as compared to children because occupational exposure is a classic risk factor for leptospirosis and involves those in direct contact with animals such as farmers, veterinarians, abattoir workers, and meat inspectors, or indirect contact such as sewer workers, miners, farmers, and fish handlers [15], however children playing with contaminated water or other things could be contributing to risk factor.

In ecological distribution, the disease distribution is significantly higher in terai (51.7%) with tropical and subtropical climate and hill region (45.5%) with subtropical and temperate climate compared to the mountainous region (2.8%) with Alpine climate (see Table: 2). This indicates the distribution of leptospirosis from tropical (Terai) to temperate (Hill) climate in Nepal with very few cases in Tundra climate (Mountainous region). This distribution of leptospirosis cases might be due to population density of different ecological region and higher chances of getting in contact of water during occupational works such as related to agriculture manual, animal handlers, forestry.

The season wise variation revealed higher leptospirosis cases in the months of July to September (rainy and post rainy seasons) in Nepal and found to be statistically associated (see Figure 1).

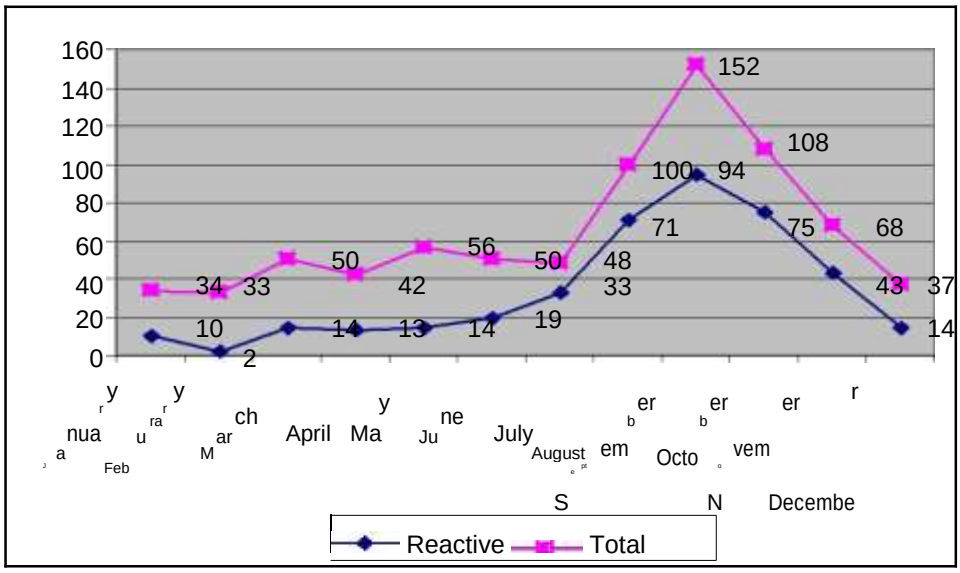

Figure 1. *Month-wise distribution of leptospirosis*

The leptospirosis cases were increasing since June (19 cases) to September (100 cases) and decreased in consecutive months with the distinct lower cases in winter months. Such pattern indicates that the transmission was higher in rainy months, as the disease is a water transmitted disease [16]. In context of Nepal, the result is likely due to farm work in rainy season where lots of people have to work in flooding and water stagnant farms. During the last 50 years, at least 17 outbreaks of leptospirosis associated with water exposure have been documented, participants of the eco-challenge in Malaysia [17], white-water rafters in Costa Rica [18], and swimmers in Brazil [19]. Other significant outbreaks with relation to water occurred in India, Argentina, Cuba, Brazil, and Nicaragua after floods and other natural disasters [17- 24].

CONCLUSION

More than 50% positivity of leptospirosis in undiagnosed cases of AES indicated that there might be large number of leptospirosis. Due attention should be given to leptospirosis which is one of the neglected tropical diseases in Nepal by provision of laboratory diagnostic facilities at all peripheral and central health institutions. It is also suggested that active surveillance on leptospirosis should be conducted on a larger population involving different occupational groups.

ACKNOWLEDGEMENT

The authers would like to acknowledge NPHL staff of virology unit carrying out their technical inputs, and WHO/IPD for their support on diagnostic kits.

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Synthesis and Characterization of ZnO Nanoparticles by Using Natural Binder and its Antibacterial Activity

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ABSTRACT

Zinc oxide nanoparticles have received considerable attention due to their unique antifungal, antibacterial and UV filtering properties, photochemical activity and catalytic activity. Inexpensive and biodegradable natural polymer used as natural binder for synthesis of ZnO nanoparticles. ZnO nanoparticles were characterized by UV-visible, XRD FTIR and SEM. Further, its antimicrobial activity against gram-negative bacteria such as Escherichia coli was also studied.

Keywords: *ZnO nanoparticles, natural binder, antibacterial activity*

INTRODUCTION

Development in synthesis of metal nanoparticles play a vital role for research in current material science due to their attention for applications in the field of electronic, information storage, optoelectronic, solar energy storage and drug delivery. Zinc oxide nanocrystalline have been found tremendous applications in the field of sensors, diagnostics, catalysis, micro-electronics and antimicrobials etc. [1-5].

In the present work, we synthesized of ZnO nanoparticles by using natural binder material i.e. Gum tragacanth. The Gum tragacanth chain contains D-galactameric acid, D- xylose, L-fucose and D-galactose. ZnO nanoparticles attributed to the generation of reactive oxygen species on the surface. The benefit of using ZnO nano as antimicrobial agent is that they contain ecofriendly elements necessary for humans.

MATERIALS AND METHODS

Chemicals

Zinc acetate, Ammonium carbonate, Dimethyl formamide, alcohol was purchased from Merck India Ltd., Mumbai. Nutrient agar was purchased from Hi-media, Mumbai. Ciprofloxacin was purchased from the local market of Pune. Gum Tragacanth was purchased from S.D. fine chemical, Mumbai. All chemicals used were of high purity. All solutions prepared in de-ionized water.

Bacterial strains

Escherichia coli was obtained from the Microbiology department, University of Pune, Pune. The strain was maintained on nutrient agar medium.

Synthesis of ZnO nanoparticles

In the synthesis of ZnO nanoparticles Gum Tragacanth 10% aqueous solution was taken in three neck flask. 0.01M Zinc acetate and 0.01M Ammonium carbonate were added to the flask containing 10% Gum tragacanth solution with constant stirring at room temperature for 48 hours. The Gum tragacanth-ZnO nanoparticles were prepared. The

obtained material was filtered, washed with methanol several times to remove the byproduct ammonium acetate, dried under vacuum for 1 hour and then calcinated in muffle furnace at 600°C for 3 hours, which gives ZnO nanoparticles.

RESULT AND DISCUSSION

UV-visible spectroscopy is an important technique to detect the formation of nanoparticles. ZnO nanoparticles were dispersed in acetone and then the solution was used for UV-visible measurement. From the Figure 1, sharp absorption peak was observed for ZnO nano materials at 312 nm. The intensity and sharpness shows that the particles are in nano sized.

The XRD spectrum pattern of the zinc oxide nanoparticles is as shown in figure 2. The diffraction peaks are observed it can be indexed to the (100), (002), (101), (102), (110), (103), (112) and (201) reflections of hexagonal structure of zinc oxide. The average particle size of the ZnO nanoparticles is calculated by using Debye Scherrer's formula, which is around 21-30 nm [1].

Figure 3 shows a broad peak of FTIR spectrum at 455/cm^{-} [2]. Figure 4 shows the rod shaped morphology of the ZnO nanoparticles. Agglomeration expected due to the high temperature [6].

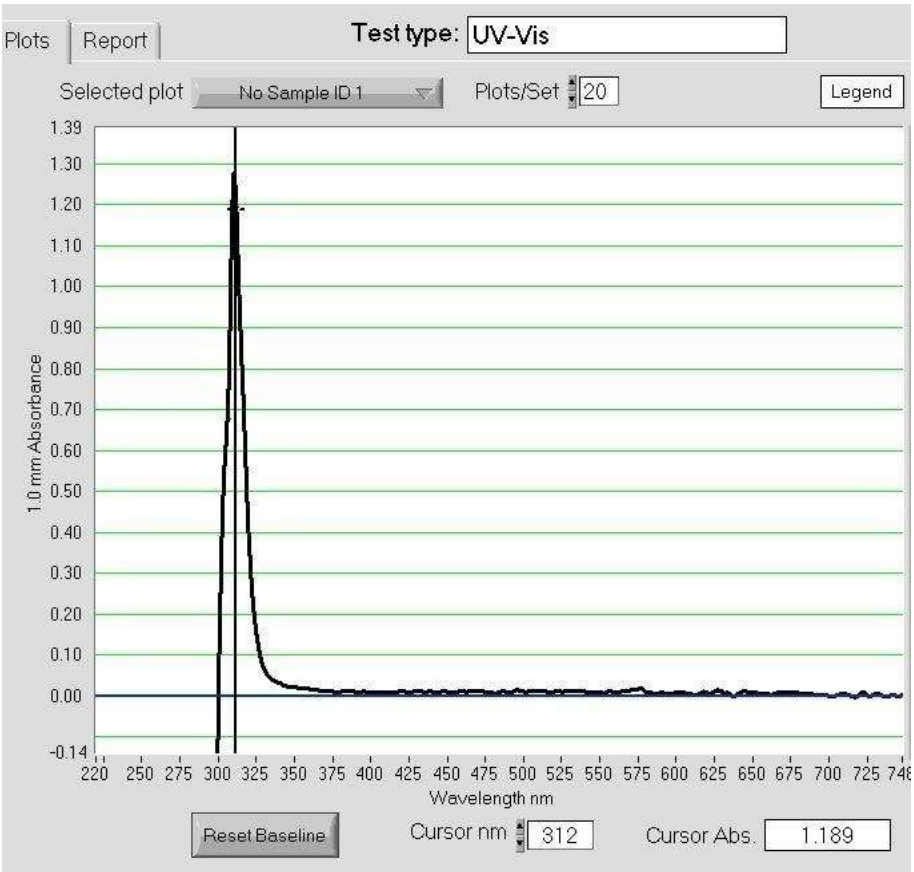

Figure 1. *UV -visible absorption spectrum for ZnO nanoparticles*

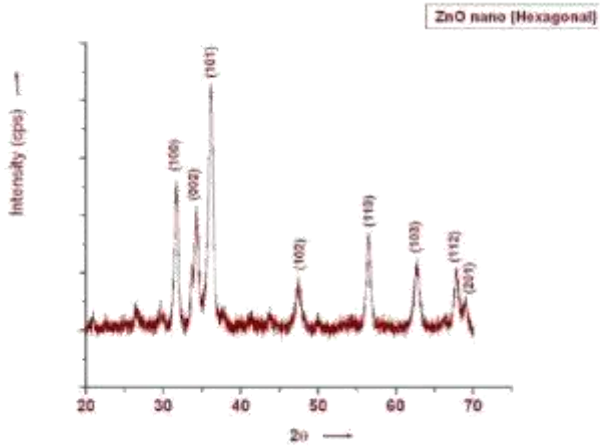

Figure 2. *XRD patterns recorded for the ZnO nanoparticles synthesized by using natural binder*

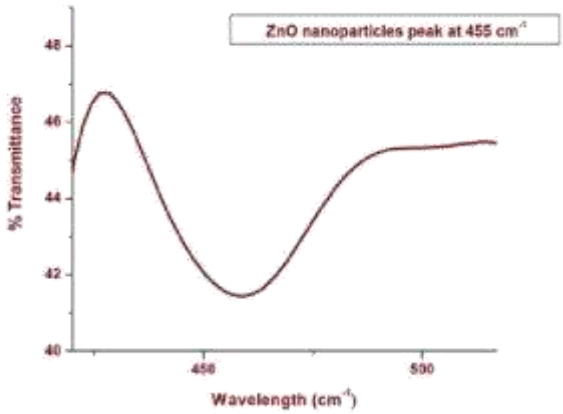

Figure 3. *FTIR spectrum of Zinc oxide nanoparticles*

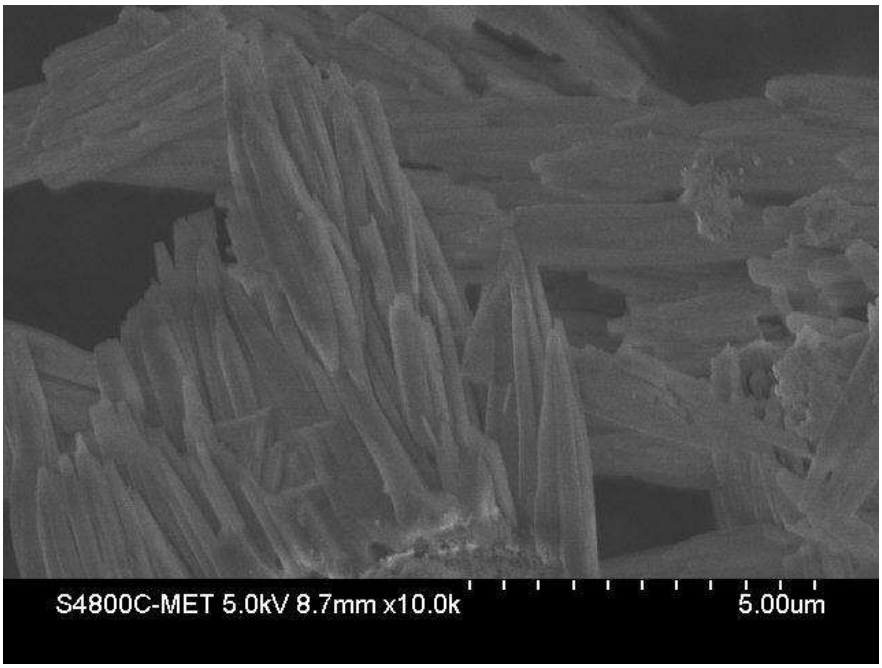

Figure 4. *Scanning electron micrograph of ZnO nanopartilces*

Antimicrobial activity

The ZnO nanoparticles showed antibacterial activity against strain of gram negative bacteria. Activity of compound was effective against *E. coli* [7].

Activity of this compound against pathogenic bacteria was determined by agar diffusion technique. Bacterial culture was incubated with ZnO nano and bulk at 37^0C for 24 hours. The results obtained after 24 hours has showed

zone of inhibition against *E.coli* (20 mm), compound was found to be effective against pathogenic bacteria and zone of inhibition was more than positive control as shown in the Figure 5.

Table 1: *Antimicrobial activity of ZnO and nano ZnO against E. coli*

Bacterial strain	ZnO bulk (mm)	ZnO nano (mm)
Escherichia coli	12	19

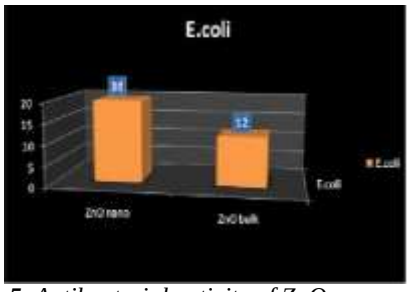

Figure 5. *Antibacterial activity of ZnO nanoparticles aginst E coli.*

Minimum Inhibitory Concentration (MIC)

Concentration of ZnO nanoparticles required to inhibit the bacteria was 50 µg/ml. Thus, the compound is not found to be bactericidal.

CONCLUSION

X-ray diffraction pattern confirms the hexagonal ZnO nanoparticles successfully synthesized. The nano ZnO makes easier to attached with the cell wall of the microorganisms causing its demolition and to the death of the cell, because nano size of ZnO. Zinc oxide posses well developed surface chemistry, chemical stability which makes them easier to interact with the microorganisms. The ZnO nanoparticles are more effective against *E. coli*.

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Multi-Drug Resistance Uropathogens among Patients Attending Janamaitri Hospital, Kathmandu, Nepal

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ABSTRACT

Urinary Tract Infection (UTI) is one of the most common infectious diseases causing worldwide. The present study has been conducted from June 2012 to September 2012, among the patients attending Janamaitri Hospital based at Kathmandu, with the objective to determine the antimicrobial susceptibility pattern of multi-drugs resistant bacterial uropathogens. Standard microbial techniques were used for culture and identification of the isolates. Kirby disc diffusion method was employed for antibiotic susceptibility testing. Out of the 831 urine specimens processed, only 12.51% (104) were culture positive. Among the culture positive cases, the most predominant were E. coli (65.38%) followed by Klebsiella Spp. (12.5%), Staphylococcus Spp (9.61%), Enterobacter Spp. (3.84%) and Morganella morganii (2.88%). Anitbiotic Susceptibility test revealed that the obtained organisms were resistance to amoxicillin (42.3%), cefixime (29.8%), cephalexin (42.3%), cotrimoxazole (21.15%) and ciprofloxacin (14.42%). This study showed that resistance patterns of pathogenic organism were high among commonly used drugs. Enterococcus, Morganella and Pseudomonas showed 100% resistance to used first line antibiotics. The present findings highlighted that constant monitoring and antibiotic susceptibility testing of the urine isolates should be performed to decrease the use of improper antibiotics.

Keywords: *Uropathogens, antibiotic resistance, Nepal*

INTRODUCTION

Urinary tract infection is a common bacterial disease, often contributes to a frequent cause of morbidity in out-patients as well as hospitalized-patients[1]. It is estimated that there are about 150 million urinary tract infections per annum worldwide[2]. UTI mainly affects children and women. The cumulative incidence rate reaches 10%. In adulthood, almost half of all women will experience at least one infection during the lifetime[3]. UTI is classified according to the dominant site of infection into infection of the lower urinary tract, cystitis and infection of the urinary tract, pyelonephritis. Bacteremia caused by microorganisms in the urinary tract constitutes nearly 15% of all cases[4]. When bacteria reach the blood stream, the disease may cause septicemia.

UTI is challenging, not only because of the large number of infections that occur each year, but also because the diagnosis of UTI is not always straight forward. UTI has to be distinguished from other diseases that have a similar clinical presentation, some UTIs are asymptomatic or present with atypical signs and symptoms, and the diagnosis of UTI in neutropenic patients (who do not typically have pyuria) may require different diagnostic criteria than those used for the general patient population. Because of these factors, much reliance is placed on laboratory tests to augment clinical impressions; even when clinical diagnoses are unequivocal, physicians may order laboratory tests to identify the cause of the infections and/or to provide isolates for anti-microbial susceptibility. It therefore comes as no surprise that the laboratory examination of urine specimen accounts for a large part of the work load in many hospital based laboratories.

Treatment of these infections is usually started empirically. Such treatment is no longer sufficient as antimicrobial resistance among pathogens causing nosocomial infections has increased in recent years[5]. Worldwide data shows that there is an increasing resistance among UTI pathogens to conventional drugs.

Resistance has emerged even to newer, more potent antimicrobial agents[6], making the therapeutic options very limited to certain antimicrobial agents like carbapenem, colistin and fosfomycin[7]. Antimicrobial resistance surveillance is necessary to determine the size of problem and to guide empirical selection of antimicrobial agents for treating infected patients

Clinical experience has indicated the presence of numerous cases of antibiotic resistance to common antibiotics by uropathogens in both developed and developing countries[8]. In many parts of Nepal, the facilities for urine culture and antimicrobial susceptibility testing are still not available, leading to improper diagnosis and irrational antibiotic treatment (e.g. self-medication) of UTI[9] . The updated knowledge and situation of the prevailing bacterial uropathogens that are multidrug resistant (MDR) is of prime importance for the proper use of antimicrobial drugs and the policy making to combat multi-drug resistance in UTIs[7].

Invariably, *Escherichia coli (E. coli)* have been found as a most common uropathogen in a number of reports worldwide. Antimicrobial therapy of UTI caused by *E. coli* is often impaired due to the resistance to commonly- used antimicrobial agents[10]. Although *E. coli* has been reported to be MDR by possessing the antibiotic resistant genes in its transferable R-plasmid[11], detection of this feature in UTI isolates from Nepal is largely unknown. The present study aimed to determine the prevalence of MDR bacterial isolates in UTI and the antibiotic resistance pattern. Our data might be informative to both of the health professionals and the scientific community, which may help to make a positive contribution to current understanding and knowledge of the situation in UTI caused by MDR bacterial pathogens.

The main objective of the study was to determine the rate of multi-drug resistant uropathogens among patients attending Janamaitri Hospital, Kathmandu, Nepal.

EXPERIMENTAL PART

Study population

The study population was drawn from patients visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal with a suspected case of urinary tract infection. Total 831 urine samples were taken for the study

Study duration: June 2012 to September 2012.

Collection of samples*:* Clean-catched mid-stream urine specimens were collected using sterile, wide mouthed glass bottles with screw cap tops. Name, Age and Sex were noted along with requisition forms. Laboratory analysis required for the present study was done according to Bailey & Scotts[12].

Sample processing

Culture

A calibrated sterile loop for the semi-quantitative method was used for the plating and it has a 4.0 mm diameter designed to deliver 0.001 ml. A loop full of the well mixed urine sample was inoculated into duplicate plates of Blood and Mac- Conkey agar. All plates were then incubated at 37^{o}C aerobically for 24 hour. The plates were then examined macroscopically and microscopically for bacterial growth. The bacterial colonies were counted and multiplied by 1000 to give an estimate number of bacteria present per milliliter of urine. A significant bacterial count was taken as any count equal to or in excess of 10,000 cfu /ml[13].

Microscopy

The urine samples were mixed and aliquots centrifuged at 5000 rpm for 15 min. The deposits were examined using both x10 and x40 objectives. Samples with >8 white blood cells/mm3 were regarded as significant pyuric[12]. A drop of well mixed urine sample was applied to a glass microscope slide, allowed to air dry, the smear is fixed, stained with gram stain, and examined microscopically under oil immersion(1000X)for the presence of >1or 5 bacteria per oil immersion field. Bacterial isolates were identified generally using a battery of tests.

Antibiotic susceptibility testing

Antibiotic susceptibility test was performed by using Kirby Bauer disc diffusion technique on Mueller–Hinton Agar (MH), as recommended by the NCCLS guidelines[13]. Antibiotic discs were obtained from Hi-Media.

Statistical analysis

Statistical analysis was done using MS-excel 2007.

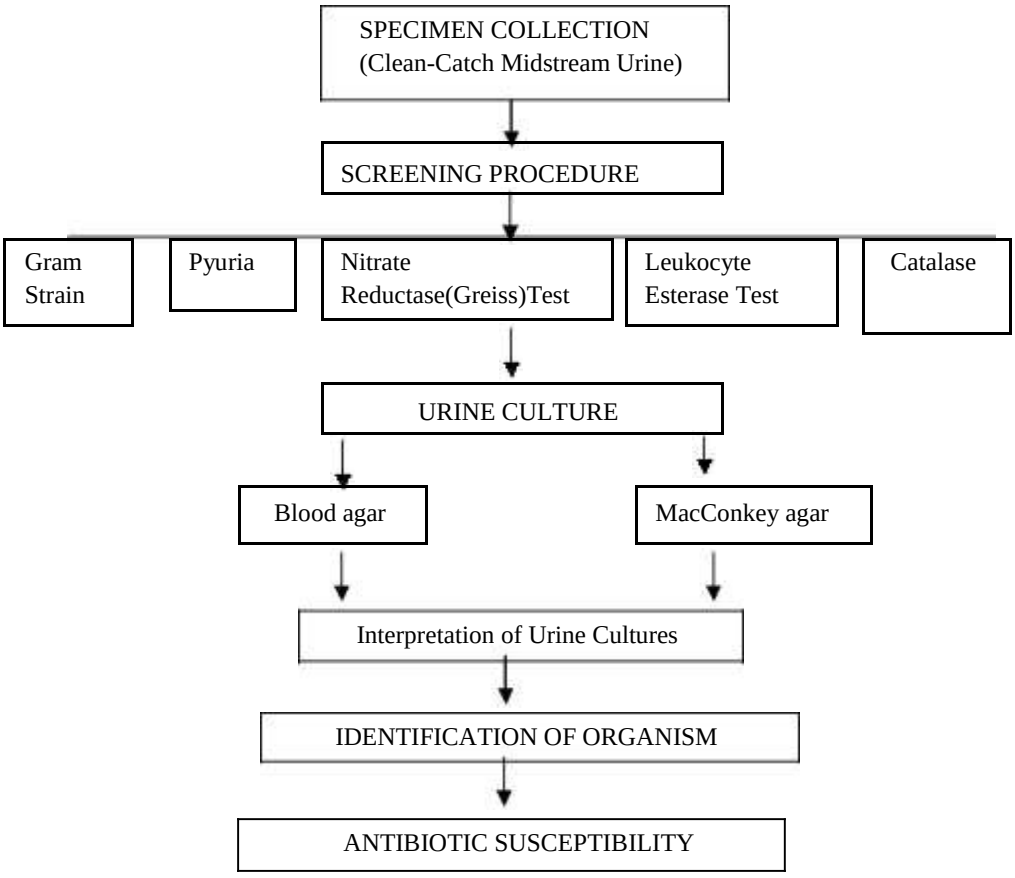

Figure 1. *Flow diagram for processing urine sample*

RESULTS AND DISCUSSION

Present study includes 831 specimens of urine collected from patients. About one third (32.69%) male and near about two third (67.31%) female had culture positive test result. Table 1 show that overall positivity was 12.51%.The prevalence of significant isolates (12.51%) in our study is similar with 14.7% of AIMS, India which is observed by Mohanty *et al*[14] but lower than the prevalence rate of 25.7% and 37.4% recorded by Kumari *et al*[15] and Rai *et al*[16] respectively. The high prevalence may be due to genuine population susceptibility because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status.

UTI was found to be more prevalent in female than in male[17]. Out of 104 positive results, 67.31% were female and 32.69% were male. This result was confirmatory with the study from Nepal, India and other countries [17, 18].Women are at greater risk for UTI than men, partly because of the relatively short, straight anatomy of the urethra. Retrograde ascent of bacteria from the perineum is the most common cause of acute cystitis in women. Host factors such as changes in normal vaginal flora may also affect the risk of UTI. Genetic factors, including expression of HLA-A3 and Lewis blood group Le (a-b-) or Le (a+b-), may also put women at higher risk for recurrent UTI. Sexually active women are at greater risk for UTI than women who do not engage in sexual intercourse. Simple hygiene habits, including voiding before and after sexual intercourse and wiping from anterior to posterior, are often advocated to decrease the risk of UTI; however, a recent review found no advantage to these behavioral techniques[19].

Table 1: *Sex wise distribution of the cases according to their culture positive test results (n=104)*

Sex	**Total Cases**	**Positive cases (%)**
Male	231	34 (32.69)
Female	600	70 (67.31)
Total	**831**	**104**

Among total 831 patients (Table 2), 171 were in- patients that were admitted to the hospital and 660 were out-patients. Out of total 104 positive cases, 16 were in- patients and 88 were out- patients. This result shows that the prevalence is higher in out patients. It might be due to out patients have specifically visited the doctors with possible signs and symptoms of urinary tract infections or other similar diseases .But the in patients may not have such symptoms and they were suffering from other diseases.

Table 2: *Patient wise distribution of the cases according to their test results (n=104)*

Patient	**Total Cases**	**Positive cases (%)**
In	171	16 (15.38)
Out	660	88 (84.62)
Total	**831**	**104**

According to age wise distribution (Figure 1), highest positive cases were found in female of age group 20-25, followed by age group of 25 -30. Among male after age 50 were the maximum positive cases. This result shows that the females of sexually active age group are more susceptible to the disease. This is as a result of shorter and wider urethra. The anatomical relationship of the female's urethra and the vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up to urethra into the bladder during pregnancy and child birth[20, 21].

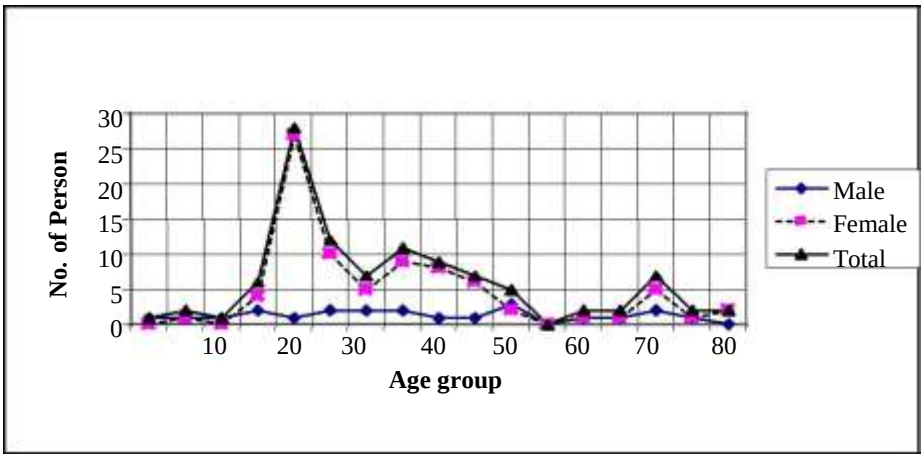

Figure 1. *Age-group wise distribution of the cases according to their culture positive result*

More than half (65.38%) cases reported as *E. coli* (See Figure 2) followed by *Klebsiella* Spp. (12.5%), *Staphylococcus* Spp. (9.61%), *Enterobacter* Spp. (3.84%), *Proteus mirabilis* (2.88%), *Morganella morganii* (2.88%) *Pseudomonas* Spp. (1.92%) and group D *Enterococci* (0.96%). The isolation rate of urinary pathogens of the present study is consistent with reports of the studies published elsewhere recently[22, 23, 24]. Isolation of *Escherichia coli* as the predominant pathogen of community associated UTI has been extensively reported in many studies[25, 26].

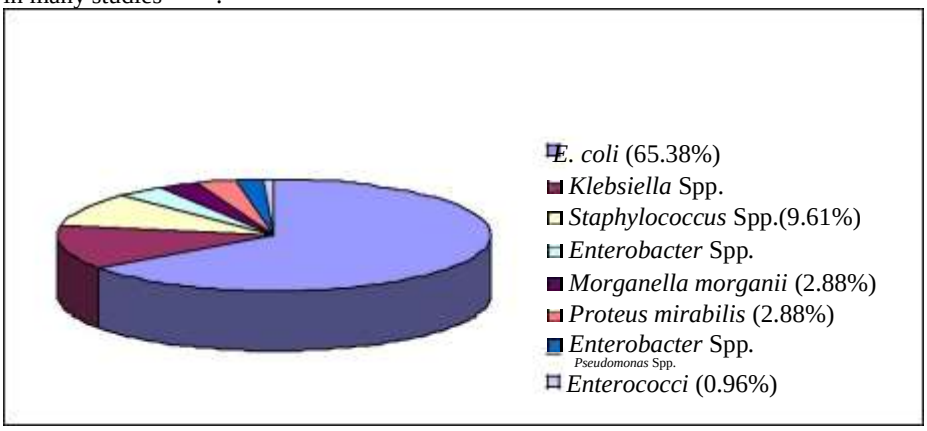

Figure2. *Types of organism found (culture positive cases)*

For the present study different drugs such as amoxicillin, cefixime, cefalexin, cotrimoxazole, flouroquinolone, nitrofurantoin, naldixic acid, erythromycin, meropenem and gentamicin were used to know the multi-drug resistant uropathogens among patients. Incidence of drug résistance (See Table 3) among the positive cases like higest resistivity of *E. coli* with cephalexin (67.65%), Staphylococci with cefixime (80%), Klebsiella with amoxicillin and cephalexin(69.23%), *Morganella morganii* with amoxicillin and cephalexin (100%), Enterobacter with cephalexin(75%), *Proteus mirabilis* with nitrofurantion(100%), Pseudomonas with amoxicillin(100%) and Enterococci with all the used First line antibiotics (100%). Resistance rates among common uropathogens to many commonly used antimicrobial agents have increased over the years and theses resistance rates vary from country to country[27]. Most of the organisms are resistant to these commonly used drugs. The results also showed that Enterobacteria were resistant to most of penicillins studied and this attributed to their ability to produce β-lactamases enzymes. Resistance to beta-lactam antibiotics in Gram-negative bacteria can be due to three mechanisms: decreased permeability of the drug into the cell, hydrolysis of the drug by β-lactamase, or decreased affinity of the target penicillin-binding proteins-PBPs[27].

Table 3: *Incidence of drug Resistance in isolated organism from urine culture*

Antibiotic	**RE S I S T A NCE (%)**							
	E. coli	*Staphylococcus* Spp.	*Klebsiella* Spp.	*Morganella morganii*	*Enterobacter* Spp.	*Proteus mirabilis*	*Pseudomonas* Spp.	*Enterococci* Spp.
Amoxicillin	37 (54.41	3 (30)	9(69.23)	3 (100)	1 (25)	0	2 (100)	1 (100)
Cefixime	25 (36.74)	8 (80)	4 (30.11)	1 (33.33)	2 (50)	0	-	1 (100)
Cephalexin	46 (67.65	4 (40)	9 (69.23)	3 (100)	3 (75)	2 (66.67)	-	1 (100)
Cotrimoxazole	19(22.94)	2 (20	6(46.15)	2 (66.67)	1 (25)	-	-	1 (100)
Ciprofloxacin	21(30.88)	1 (10)	6 (46.15)	2 (66.67)	0	0	0	0
Nitrofurantoin	8 (11.76)	-	4 (30.77)	2 (66.67)	0	3 (100)	1 (50)	-
Nalidixic Acid	29 (42.65)	-	7 (53.34	1 (33.33)	1 (25)	2 (66.67)	1 (50)	-
Erythromycin	-	3 (30)	-	-	-	-	-	1 (100)
Meropenem	-	-	-	-	1 (25)	-	1 (50)	-
Gentamicin	-	-	-	-	-	-	1 (50)	1 (100)

Our findings also showed that the multi drug resistance patterns of organism (See Table 4) were found highest in Pseudomonas, Morganella and Enterococcus i.e. 100%, followed by Klebsiella 76.92% and so on. Our study shows that all the organisms isolated were multi drug resistant. In this condition it is being very difficult to select the correct effective antibiotic. Antibiotic susceptibility test is very essential before prescribing antibiotic in order to decrease the bad effect of wrong antibiotics

Table 4: *Multi drug resistance pattern of the organism (n=104)*

Name of organism	**Total No.**	**MDR strain (%)**
E. coli	68	38 (55.88%)
Klebsiella Spp.	13	10 (76.92%)
Enterobacter Spp.	4	1 (25%)
Pseudomonas Spp.	2	2 (100%)
Morganella morganii	3	3 (100%)
Proteus mirabilis	3	2 (66.67%)
Staphylococcus saprophyticus and *Staphylococcus aureus*	10	7 (70%)
Enterococcus Spp.	1	1 (100%)

CONCLUSIONS

This study showed that the highest percentage of positive cases of urinary tract infections was found in female of the age group 20-30. The multi drug resistance pattern among commonly used drugs is also very high among the isolates. *E. coli* is the most frequently found organism. The findings of this research suggested the need for constant monitoring and surveillance of susceptibility of specific pathogens to commonly used anti-microbial agents to prevent emergence of further resistance.

ACKNOWLEDGEMENTS

The authors would like to sincerely thank Janamaitri Hospital, Balaju, organizing committee of ICIDN-2012, and the Department of Microbiology, Kantipur College of Medical Sciences, Sitapaila, Kathmandu.

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Preventive Practices on Bird flu among Poultry Farm Workers in Nepal: A Cross Sectional Study

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ABSTRACT

Avian Influenza- commonly called "bird flu" possesses a threat to global public health. Most cases of avian influenza infection in humans have resulted from contact with infected poultry or surfaces contaminated with secretion/excretions from infected birds. This study aimed to find out the measures practiced by poultry farm workers for the Prevention of bird flu. Study was cross-sectional based on primary data obtained through interviewing semi-structured questionnaire and applying non-participatory observation among total 54 poultry workers from total 18 registered poultry farms at Pokhara Sub-metropolitan, Nepal.
Among the 87% of poultry farmers who had heard of bird flu, 72% had knowledge on transmission of bird flu. 17% of respondents were unknown about any kind of preventive measures on bird flu. Hand washing practices before entering the farm is 48.1% and after leaving the farm is 100%. 35.1% of total respondent use gloves, 74.1% use masks, etc. Only 13% had taken trainings for managing the poultries.
Higher educational status, longer duration of involvement and profession as poultry owner directly influence respondents to know about bird flu and apply preventive measures. Proper orientation, trainings on preventive measures and management of poultries is essential to lessen the risk of bird flu.

Keywords: *Bird flu, poultry workers, Pokhara sub-metropolitan, preventive practices, Nepal*

INTRODUCTION

Avian Influenza (AI) - commonly called "bird flu" is a highly contagious viral diseases caused by influenza viruses (H5N1) affecting several species of food producing birds as well as pet birds and wild birds. Some of these AI viruses have also been reported to cross the species barrier and cause disease or subclinical infections in humans and other mammals [1]. Infection with avian influenza in domestic poultry has been classified in two forms of disease that are distinguished by low and high extremes of virulence. Highly pathogenic avian influenza (HPAI) virus spreads rapidly, may cause serious disease and result in high mortality rates (up to 100% within 48 hours). The low pathogenic avian influenza (LPAI) can also cause outbreak in poultry but with mild disease that may be undetected or no symptoms at all in some species of birds[2, 3].

Human H5N1 influenza infection was first recognized in 1997 when this virus infected 18 people in Hong Kong, causing 6 deaths during poultry outbreak [1, 4] .This avian virus has spread from Asia to Europe and Africa since its widespread re-emergence in 2003 and 2004 and has become entrenched in poultry in some countries, resulting in millions of poultry infections, several hundred human cases, and many human deaths. Outbreaks in poultry have seriously impacted livelihoods, the economy and international trade in affected countries [1].

Worldwide, WHO has reported 476 laboratory confirmed human cases and 283 deaths since the first case was reported in 2003 [5]. South-East Asia Region accounts for 190 cases (39.9%) and 152 deaths (53.7%). Of these, Indonesia has reported 163 laboratory confirmed cases and 135 deaths since July 2005, Thailand has reported 25 cases with 17 deaths between December 2003-2006; in 2008 Bangladesh reported its first and only human case of avian influenza in a 16-month-old male from Komalapur, Dhaka and in late 2007, Myanmar reported its first human case in a seven year old girl from Shan States (East). Both cases in Bangladesh and Myanmar have since recovered [5].

Nepal has detected the first cases of bird flu in January 16, 2009 [6] . Since then repeatedly outbreak of bird flu has been reported in different districts. Most of these cases (96% of the disease) of avian influenza infection in humans

have resulted from people having direct contact with H5N1 infected poultry or surfaces contaminated with secretion/excretions from infected birds. It was also found that out of 10 human infected from H5N1 virus, 6 would have died [7].

Currently, highly pathogenic avian influenza (HPAI) A (H5N1) virus is considered endemic among poultry in six countries (Bangladesh, China, Egypt, India, Indonesia, and Vietnam). Sporadic outbreaks have occurred among poultry in other countries [8].

Poultry plays a major role in transmitting bird flu between birds and community as humans are directly involved in this industry with birds. It is supposed that when bird flu take the epidemic form it is difficult to control and afford for the cases [2, 4]. Therefore, study on preventive measure used among poultry workers helps to take the necessary measures for the improvement of prevention practices and helps in making and analyzing the strategies of bird flu. Thus, study was conducted to assess practice of preventive measures on bird flu among poultry farm workers of Pokhara Sub metropolitan.

METHODOLOGY

Participants and procedures

The present cross-sectional study was conducted among the poultry farm workers at Pokhara sub-metropolitan, Nepal. All poultry workers (n=54) involved during study time at all the registered poultry farms (i.e., 18 poultry farms) at Pokhara sub metropolitan were included in the study. The study was based on primary data which was obtained through interviewing pre-tested semi-structured questionnaire with the workers and applying non-participatory observation of poultry farms. Variables related to socio demographic profile, knowledge on preventive measures were included in the questionnaire, practiced preventive measures during the study time was assessed with observational checklist and operational definition of terms included in preventive measures were defined. Polite, simple and local language was used while asking questions and informed verbal consent was taken from each of the respondent. Poultry workers not giving consent to participate and absent during the visit to the farm were excluded from the study. Pre-testing of the questionnaire was done in similar situation and necessary modification in the test instrument was carried out. The study was carried out for the period of six months i.e., September 2010 – January 2011. Ethical approval for conducting study was taken from the college and poultry farms prior to the study.

Statistical analysis

The collected data was checked for its completeness, correctness and internal consistency to exclude missing data. Coding, classification and tabulation was done for facilitating analysis and interpretation. The data collected were analyzed and processed through the computer using SPSS 16 version for windows. Percentage, mean and standard deviation were calculated where ever necessary to draw out observation and meaningful conclusions.

RESULTS AND DISCUSSION

Socio-demographic findings

Among the total 54 respondents, majority i.e., 43(79.6 %) were males and 11(20.4 %) were females; maximum respondents belong to age group 20 -39 years i.e. 64.8% (Mean age= 33; standard deviation=11.255). Respondents of different ethnicity were found to be involved in the poultry farm. Of the total respondents Brahmin and Chhetri occupies 46.3%, Janjati occupies 42.6% and Dalit occupies 11.1% .Majority of respondents were of Hindu religion i.e. 75.9% followed by Buddhist religion with 24.1%. Nearly ¾ (72.22%) were employed workers while remaining 27.78% were owners of the poultry farms. The study found that 42.5% respondents had completed their primary level education, 16.7% completed higher secondary, 16.7% completed lower secondary and 14.8% were illiterate. 29.6% of the respondent has experience in the poultry work for more than 6 months and less than 1 year while 16.7% had experience more than 10 years. From the findings it was revealed that educational status and work experience was more in owners compared to employed workers.

Knowledge on preventive measures

Among the 87% of poultry farmers who had heard of bird flu, 60% had knowledge on bird flu, 72% had knowledge on transmission of bird flu and 17% of respondents were unknown about any kind of preventive measures on bird flu. It was found that with the increase in educational status i.e. respondent with secondary and higher secondary level had more knowledge about bird flu 88.9% and 100 % respectively.100% respondents with more than 10 years experience had known about bird flu. The respondent on knowing about bird flu is increasing with the increase in duration of involvement. Similarly 100 percent owners knew about bird flu while only 82.1 percent poultry workers knew about bird flu (Table 1).

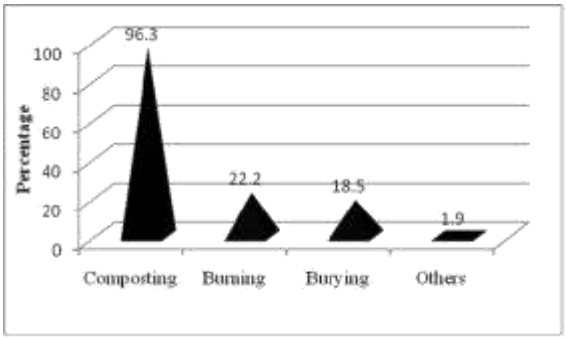
Table 1: *Comparison of Knowledge with demographic variables (n=54)*

Variables	known about bird flu				Total	
	Yes		No			
	N	%	N	%	N	%
Educational Status						
Primary	22	95.7	1	4.3	23	100
Lower secondary	3	60	2	40.0	5	100
Secondary	8	88.9	1	11.1	9	100
Higher secondary	9	100			9	100
Illiterate	5	62.5	3	37.5	8	100
Total	47	87	7	13	54	100
Duration of involvement						
Less than a year	12	75	4	25	16	100
1-2 yrs	8	80	2	20	10	100
2-5 yrs	11	100			11	100
5-10 yrs	7	87.5	1	12.5	8	100
More than 10 yrs	9	100			9	100
Total	47	87	7	13	54	100
Profession						
Owner	15	100			15	100
Worker	32	82.1	7	17.9	39	100
Total	47	87	7	13	54	100

Where, N=Number of respondent

Practices related findings

The practice of cleaning farm after one cycle is completed was found on 90.7% respondents. Similarly, 90.7% used to dispose poultry farm waste separately outside the farm and remaining 9.3% used to dispose separately within the farm. 96.3% responded that they practice composting method, 22.2% practiced burning, 18.5% practiced burying and 1.9% practiced other (putting in bio gas) for disposal of poultry wastes (Fig. 1). 48.1% used to dispose liquid wastes in separate pit, while 24.1% dispose in public drain via home drain and 27.8% disposed in others source (field, biogas) (Fig. 2). Disposal of animal carcasses by burying was practiced by 85 % while 15% used to dispose the animal carcasses in fish pond, giving it to pig, and putting in biogas. Medication of sick poultries was practiced by 90.7% while 1.9% used to sell sick poultries. It was found that the distance of farm from the residential area was 678 meters in average, maximum farm i.e. 55.6% were at the distance less than 200 meter from residential area.



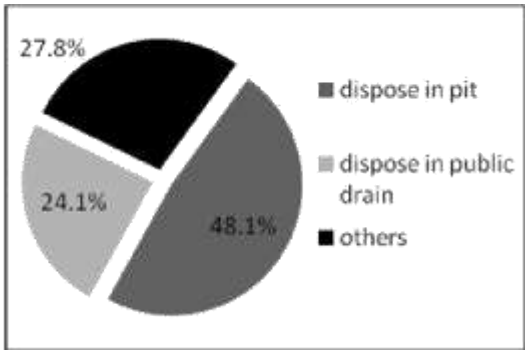


Figure 1. *Way of disposing poultry farm wastes* ***Figure 2.*** *Way of managing liquid wastes (Multiple responses)*

Practice of vaccination of poultries was among all the respondents. The practice of spraying in the body was present in 33.3% while the practice of spraying at the farm is 88.9% (Fig. 3).The spray used for spraying in the body was antiseptic solution and in the farm was mostly chemicals.

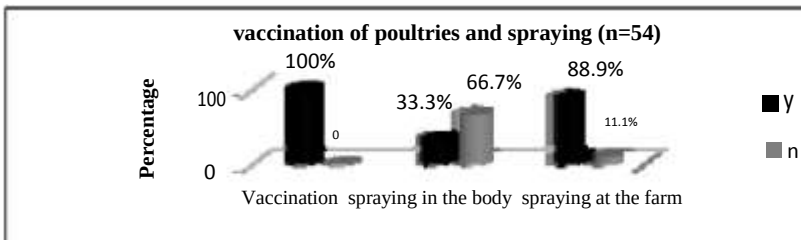


Figure 3. *Vaccination of poultries and spraying*

Practice related variables that are observed during data collection are shown in tabular form below (Table 2). Only 13% involved in the profession had taken training for managing the poultries in poultry farms.

Table 2: *Practice related variables (n=54)*

Protective measures	**Percentage**
Hand washing Practice	
Before entering the farm	48.1
After leaving the farm	100.0
Use of gloves	31.5
Use of Masks	74.1
Use of Boots	40.7
Use of Aprons	64.8
Presence of Soap & water	98.1
Presence of Antiseptic Solvent	1.9
Water logged around the poultry farm	24.1
Vaccination of poultries	100.0
Separate yard for disposing poultry waste	100.0

The present study aimed to assess the measures practiced by poultry farm workers for the prevention of bird flu. The practice related behavioral findings for the prevention of bird flu conducted in similar study at Rupandehi district of Nepal reveals same percentage of practice for hand-washing and use of gloves while the practice of use of masks

was ¾ times more, use of boots was nearly six times more and practice of spraying and disinfecting farm was twice more in the present study findings [6]. The findings of the study that practice of prevention measures increases along with the higher educational status and working in the poultry farm for longer duration was similar to many studies [6,9,11]. This could provide scientific support to assist the government in developing strategies and health-education campaigns to prevent AI infection among the poultry workers. Knowledge regarding bird flu, its transmission and prevention practice was similar and the level of implementing already known preventive measures was found low among the poultry farmers along with inadequate knowledge about bio-security which is similar to present study [10]. Despite the strict rules to be followed about preventive measures, employed workers do not follow unless guided by the owners was similar to the findings of present study [10] . Study conducted on Hong Kong, China in 2009 reported the similar findings as the present study which includes low-to-moderate levels of compliance with hand hygiene and other preventive measures [11]. Lack of proper orientation and training before and during the work contributed as the barrier for practicing the preventive measure of present study was similar with the findings[11]. The study conducted in Vietnam among high -risk population to assess the impact of educational intervention concerning awareness and behaviors relating to avian influenza (H5N1) concluded increased awareness of H5N1 and increased reliance on local health care workers which is the finding similar to other studies [12]. Therefore, these finding supports the findings, and conclusion of this study that designing and implementing of educational and awareness programs to poultry farmers is utmost essential.

CONCLUSION AND RECOMMENDATION

The difference in knowledge and practiced behavior shows that poultry workers are not aware of themselves about the preventive measures. They are guided either by owner of poultry or there long experience. Higher educational status, longer duration of the involvement in the profession and the profession as owner rather than worker were found to be the influencing factors for practicing the preventive measures. Knowledge and practice can be bridged by designing and implementing the educational and awareness programs on bird flu, proper orientation and trainings on the management of poultries and preventive measures against the bird flu to lessen the risk of bird flu in the community and nation as a whole. These programs need to be launched to the workers along with owners. Simple but highly effective techniques such as: use of personal protective measures, hand washing practices before entering and after leaving the farm with soap and water, use of gloves, etc are highly recommended.

ACKNOWLEDGEMENT

The author thanks the participating respondents, staffs of District Public Health office, Pokhara, Kaski and Pokhara sub-metropolitan office for their co-operation.

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Intravascular Catheter Related Infections and Antibiotic Susceptibility Pattern of the Isolates from Catheters used in Different Wards of Shahid Gangalal National Hospital, Kathmandu

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ABSTRACT

Intravascular catheter related infections (CRI) is one of the major cause of hospital acquired infection in the world. With the aim to determine the incidence of CRI, its causative agents and antimicrobial effects of some of the commonly prescribed drugs in Nepal, a cross-sectional study was carried out from June 2011 to July 2012 among the patients using catheters at different wards of Shahid Gangalal National Hospital, Kathmandu. Extraluminal Maki's roll over plate method (semiquantitive method) and endoluminal catheter flush culture (vortexing method) were used for processing of catheter samples. Of the 197 catheters processed for culture, only 15.22% (30) showed CRI. Local colonization was reported in 8.62% of the samples whereas 6.09% had exit-site infection and 0.50% were bacteremia. In contrast, 15.22% showed phlebitis. Higher rate of CRI was reported in patients admitted at intensive care unit (56.66%) of the hospital whereas medical intensive care unit, critical care unit and new medical ward showed a similar infection rate of 10 %, and both the new surgical ward and general ward A had least infection (3.33%). Most common isolate was Staphylococcus aureus (33.33%) followed by Staphylococcus epidermidis (23.33%) and Pseudomonas aeruginosa (13.33%). Other bacterial pathogens isolated were Escherichia coli (6.66%), Acinetobacter spp. (6.66%), Klebsiella pneumoniae (3.33%), and Streptococcus pneumoniae (3.33%). Ten percent of the total isolates were the fungal organism-Candida albicans.
Within the Gram positive bacterial isolates, Staph. aureus and Staph. epidermidis showed 100% sensitivity to vancomycin whereas Streptococcus pneumoniae was susceptible to all the antibiotic tested (…….). Among the Gram negative bacteria, E. coli was 100% sensitive to ceftazidime. Klebsiella pneumoniae was sensitive to both meropenem and ceftazidime whereas P. aeruginosa were only sensitive to meropenem. None of the Acinetobacter spp. isolates were resistant to piperacillin-tazobactam. These findings necessitates a regular monitoring, periodic surveillance and appropriate infection control policy for reducing the incidence of antibiotic resistant nosocomial infections among the patients using intravenous catheters.

Keywords**:** *Intravascular catheter related infections, hospital, Kathmandu*

INTRODUCTION

Intravascular catheter related infection (CRI) is the infection due to inserted catheters at the insertion site or bacteremia by dissemination of organisms through infected catheters. Intravascular catheter-related complications range from local exit site or tunnel infections to serious, death causing bacteremias [1].

Intravenous access in the intensive care unit (ICU) and other wards setting is now routine for the administration of fluids, blood products, drugs, parental nutrition and haemodynamic monitoring [2]. Use of intravascular catheter in acutely or chronically ill hospitalised patients may be peripheral vascular catheters (PVC), arterial catheter or different central venous catheter (CVC) that can be single, double, or triple-lumen [3]. Among these catheters most used are cannulae for peripheral use [4]. CVCs are inserted centrally (jugular, subclavian or femoral) [5].Whereas, PVC are inserted on the lower extremities or upper arm [6]. Unfortunately, these access devices are responsible for iatrogenic infections especially bloodstream infection originating from colonization of the device.

Intravascular catheters are an increasingly important cause of nosocomial infections and the reported incidence of CRI is variable and ranges from 0 to 18% [7, 1]. Vascular catheters intrude the protective barrier of the skin, thus, potent microorganisms gain direct access to the bloodstream [8].

The use of intravascular catheters has been related with local and systemic infectious complications, including local site infection, CR-BSI and other metastatic infections such as brain abscess, osteomyelitis and endophthalmitis. The majority of serious CRI develop from CVC inserted in patients with critical illnesses [9]. A bacteremia was considered catheter related if the same microorganism was isolated from the catheter tip culture and from blood collected by venipuncture [10]. Blood stream infection is more frequent with CVC than PVC [4]. CRI are more associated with immunocompromised patient [11]. *Staphylococcus aureus*, coagulase negative *Staphylococcus epidermidis* are frequently isolated bacteria. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Acinetobacter* spp, *Candida albicans* are also isolated [12, 13].

The National Nosocomial Infection Surveillance System (NNIS) in the United States has reported that most nosocomial bloodstream infections in ICU are associated with indwelling intravascular devices[14]. It is estimated that infections associated with the use of intravascular catheters represent around 10-20% of all nosocomial infections [15]. Similarly, there is unnecessary removal of catheters in suspect of bacteremia and the infection is not confirmed until the tip is cultured must which causes unwanted pain and extra burden of cost for patient [16].

The skin is the main source for short-term catheter colonization and infection and the bacteria that are in the patient's skin migrate over its surface, colonizing the distal extremity, which results in infection [17] . The risk of infection is aggravated when catheters are likely to be contaminated with microorganisms from the medical practitioner or environment due to poor hygiene [18].

Infection of triple lumen catheters is three times greater than that of single lumen catheters [19]. Device insertion (and reinsertion) is unpleasant for patients, requires skilled and available clinical staff [20]. Thus, CRI remains one of the most common causes of the nosocomial infection and remains the important problem, in both economics and human terms, and should not be undervalued [21]. Although, most of the CRI like local infection resolve spontaneously with the removal of infected catheter within some days, CR-BSI may occur in 3 to 8% of inserted catheters, if not treated properly, leading to the first cause of nosocomial infection [22].

Proper diagnosis and treatment of the infection can reduce the incidence of nosocomial infection. The main aim of this study is to isolate the common etiological agents of CRI (local infection and bacteremia) in the patients admitted in ICU and wards of a hospital, determine the antibiotic susceptibility pattern of those isolates and also determine the effectiveness of commonly used antibiotics on the isolates. Moreover, improper use of antimicrobials has resulted in the rapid rise of resistant organisms [23]. It is, hence, important to determine the pattern of modern day isolates from catheter related infection and deduce the organism's antibiotic susceptibility pattern so that it would be easier for the clinicians to plan a general outline of treatment for patients with catheter related infection. This study will target to draw a general picture of the common causative organisms of catheter related infection and their antibiotic susceptibility patterns and help to know further about this infection. This study will also be followed to determine the efficiency of commonly used antibiotics for inhibiting the organisms isolated from the catheter and hence attribute to reduce nosocomial infection.

EXPERIMENTAL PART

Collection of sample

The skin was cleaned with 70% alcohol prior to catheter removal. The catheter was held at the proximal end and carefully removed from the patient with a sterile instrument, taking care to avoid contact with the skin. The distal end was held over a sterile tube, and the distal segment (5cm for central catheters and 2-3 cm for peripheral short catheter) of the catheters was cut with sterile scissors[24, 25]. The catheter segments were transported to the laboratory in sterile, dry containers [26].

Sample transportation

The samples, thus collected, were transported to the microbiology lab within 2 hours.

Sample processing

Extraluminal Maki's roll over plate method (semiquantitive method) and endoluminal catheter flush culture (vortexing method) were used for processing the collected catheter samples.

Extraluminal Maki's roll over plate method was performed as follows: Using sterile forceps, the catheter tip was removed from the transport tube and laid on a blood agar plate. The tip was rolled back and forth across the entire surface of a blood agar plate using sterile forceps and exerting slight downward pressure [26].

For endoluminal catheter flush culture, 1 ml of sterile brain heart infusion broth (BHI) with the catheter tip was vigorously vortexed for 1 minute after which aliquot of 0.1ml of suspension was placed with the help of sterile syringe, and streaked onto the Mac Conkey Agar (MA) and Blood Agar (BA) using a 4 mm inoculating loop [16, 24] and incubated at 37ºC aerobically (in a candle jar at 37ºC, for suspected streptococci).

After incubation, the plates were observed for the growth of the bacteria. The colony characteristics of the isolated bacteria were noted. Gram staining of the isolated bacterial colonies was performed. Identification of the isolates was done on the basis of their morphology and biochemical tests (For *S. aureus* slide coagulase test, tube coagulase test and catalase-oxidase test were done). Similarly, germ tube test were done for *Candida albicans*. Antibiotic susceptibility test was carried out according to modified Kirby Bauer Disc Diffusion Method as recommended by the Clinical Laboratory Standards Institute [27].

Blood sampling and culture: 5ml blood was collected within 48 hours of catheter collection under aseptic precautions from peripheral vein of the patient and inoculated in a bottle containing 50ml BHI broth and incubated at 37ºC. After 48 hours of incubation blood were subcultured on MA and BA. This process was repeated every 24 hours for 7 days if organisms were not isolated [26].
[Pictures of Antibiotic susceptibility test for *S. aureus*, growth of *S. aureus* on BA, germ tube test for *C. albicans* and slide and tube coagulase test for *S. aureus* were taken in Microbiology Laboratory of Shahid Gangalal National Hospital]

RESULTS AND DISCUSSION

Among the total 197 catheters studied, 63.45% (125) were peripheral and 36.54% (72) were central catheters. The most common site of insertion for peripheral catheters was arm veins, whereas for central catheters it was the subclavian vein. The infection and colonization rates for all the 197 intravenous catheters was 15.22% (n = 30) and 8.62% (n = 17) respectively. Whereas, local infection and phlebitis was reported in 6.09% (n= 12) and 15.22% (n= 30) cases respectively. Phlebitis was seen in 30 patients (that is 30 peripheral catheters) with same signs and symptoms as infection but no organisms were isolated. Only one case (0.50%) associated with peripheral catheter colonization showed bacteremia and septicemia. Infection associated with long duration catheter placement (6-11 days) was proportionally higher (56.66%) than in short duration catheter placement (2-5 days) which was 43.34%. Gram positive organisms had relatively high infection rate compared to Gram negative organism and fungi (Table 5). The p value for the duration of catheter placement and the growth of organism in this study was 0.111 (>0.05).

Infection rate of 10% was seen in MICU (medical intensive care unit), CCU (critical care unit) and NMW (new medical ward) whereas 6.66% in DBC (double bed cabin) and 3.33% in NSW (new surgical ward) and GWA (general ward A) respectively. Most infections were associated with intensive care unit (ICU) that is 56.66% (see Table 3). The most common isolate obtained was *Staphylococcus aureus* (33.33%) followed by *S. epidermidis* (23.33%), *P. aeruginosa* (13.33%), *E. coli* (6.66%), *Acinetobacter* spp (6.66%), *K. pneumoniae* (3.33%), and *Streptococcus pneumoniae* (3.33%). *Candida albicans* accounted for 10% of the total isolates (see Table 4).

***Table 4**: Distribution of organisms in CRI*

Organisms	Total	Percent (%)
Staphylococcus aureus	10	33.33
Staphylococcus epidermidis	7	23.33
Pseudomonas aeruginosa	4	13.33
Escherichia coli	2	6.66
Acinetobacter spp.	2	6.66
Klebsiella pneumoniae	1	3.33
Streptococcus pneumoniae	1	3.33
Candida albicans	3	10.00

The number of organisms isolated was higher in patients using multiple lumen catheters (53.33%) which might be attributed to use of these catheters for different purposes. In contrast, there was relatively low microbial load (46.66%) in patients using single lumen catheter. In multiple lumen catheters, Gram positive bacterial infection rate was higher followed by Gram negative bacteria and fungi. Even though single lumen catheter also showed similar trends for both Gram positive and Gram negative bacteria, no fungal species were isolated. The p value for the number of lumen in catheter and the growth of organism in this study was 0.142 (>0.05).The maximum number of infection (5 out of 30) was seen in the age groups 50-59 and 60-69 years. The p value for the age-wise distribution of the growth of bacteria in this study was 0.973 (>0.05).

Table 7: *Antibiotic susceptibility pattern of Gram positive bacterial isolates*

Antibiotics tested	**Category**	*P. aeruginosa*(n=4) (%)	*Acinetobacters*p(n=2)(%)	*E. coli* (n=2)(%)	*Kl. pneumoniae* (n=1) (%)
Ampicillin (Amp)	Resistant	0	0	2(100)	0
	Intermediate	0	0	0	0
	Sensitive	0	0	0	0
Gentamicin (Gen)	Resistant	1(25)	1(50)	1(50)	1(100)
	Intermediate	2(50)	0	0	0
	Sensitive	1(25)	1(50)	1(50)	0
Ciprofloxacin (Cip)	Resistant	3(75)	1(50)	1(50)	1(100)
	Intermediate	0	0	0	0
	Sensitive	1(25)	1(50)	1(50)	0
Cotrimoxazole (Cot)	Resistant	0	0	1(50)	0
	Intermediate	0	0	0	0
	Sensitive	0	0	1(50)	0
Meropenem (Mrp)	Resistant	0	1(50)	0	0
	Intermediate	0	0	0	0
	Sensitive	4(100)	1(50)	0	1(100)
Piperacillin-tazobactam (pip/taz)	Resistant	2(50)	0	0	0
	Intermediate	0	0	0	0
	Sensitive	2(50)	2(100)	0	0
Ceftazidime (CAZ)	Resistant	1(25)	1(50)	0	0
	Intermediate	0	1(50)	0	0
	Sensitive	3(75)	0	2(100)	0
Amikacin (Ak)	Resistant	0	1(50)	0	0
	Intermediate	1(25)	0	0	1(100)
	Sensitive	3(75)	1(50)	0	0

Table 6*: Age-wise distribution of CRI*

Age group	**Sex**		**No. of catheter samples taken under study**	**No. of culture positive sample**	**p value**
	Male	Female			
< 1yr	9	9	20	4	>0.05
2-9	9	9	23	3	
10-19	6	7	18	1	
20-29	5	5	14	3	
30-39	6	2	10	2	
40-49	6	7	13	2	
50-59	11	17	33	5	
60-69	14	19	36	5	
70-79	10	6	16	4	
80-89	5	2	14	1	
Total	**81**	**83**	**197**	**30**	

Table 8: *Antibiotic susceptibility pattern of Gram negative bacterial isolates*

Antibiotics tested	**Category**	*S. aureus*(n=96)(%)	***S.epidermidis***
Amoxicillin (Amx)	Resistant	4(40)	4(57.1)
	Intermediate	0	0
	Sensitive	6(60)	3(42.9)
Gentamicin (Gen)	Resistant	3(30)	1(14.3)
	Intermediate	1(10)	3(42.9)
	Sensitive	6(60)	3(42.9)
Ciprofloxacin (Cip)	Resistant	2(20)	5(71.4)
	Intermediate	2(20)	1(14.3)
	Sensitive	6(60)	1(14.3)
Cotrimoxazole (Cot)	Resistant	6(60)	2(28.6)
	Intermediate	3(30)	5(71.4)
	Sensitive	1(10)	0
Vancomycin (Van)	Resistant	0	0
	Intermediate	0	0
	Sensitive	10(100)	7(100)
Cephalexin (CN)	Resistant	8(80)	5(71.4)
	Intermediate	1(10)	1(14.3)
	Sensitive	1(10)	1(14.3)
Cefoxitin (Cfx)	Resistant	6(60)	5(71.4)
	Intermediate	1(10)	0
	Sensitive	3(30)	2(28.6)

Antibiotic susceptibility pattern of the isolates showed that vancomycin was the most effective drug against *S. aureus* and *S. epidermidis*. Other drugs such as gentamicin, amoxicillin, ciprofloxacin were also effective against *S. aureus* as evidenced by a susceptibility rate of about 60%. Besides these, susceptibility rates of 30% against *S. aureus* were observed with cefoxitin. *S. epidermidis* were 42.9% susceptible to gentamicin and amoxicillin. Meropenem was the most effective against *P. aeruginosa* and piperacillin/tazobactam was most effective against *Acinetobacter* spp. of Gram negative bacteria. Similarly, Ceftazidime was found to be the most effective drug against *E. coli* isolates. *Klebsiella pneumoniae* was sensitive to meropenem while resistant to gentamicin, ciprofloxacin and cephalexin and intermediate to amikacin. The antibiotic susceptibility test of *Streptococcus pneumoniae* showed that it was susceptible to all the antibiotics tested.

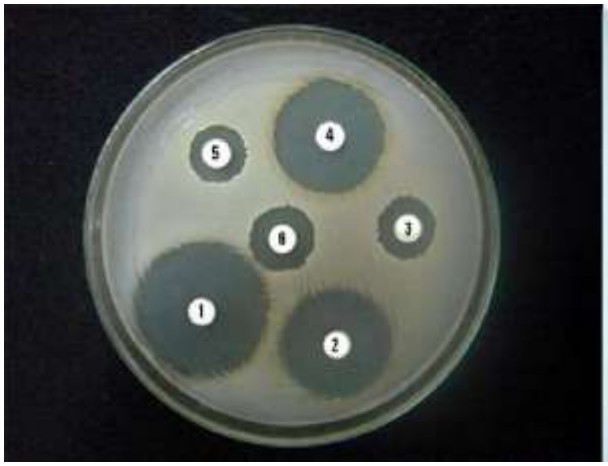

Figure 1. *Antibiotic susceptibility test of S. aureus on Muller Hinton Agar (1. Ciprofloxacin 2. Vancomycin 3. Cefoxitin 4. Co-trimoxazole 5. Cephalexin 6. Amoxicillin)*

Figure 2. *Blood agar plate showing growth of S. aureus*

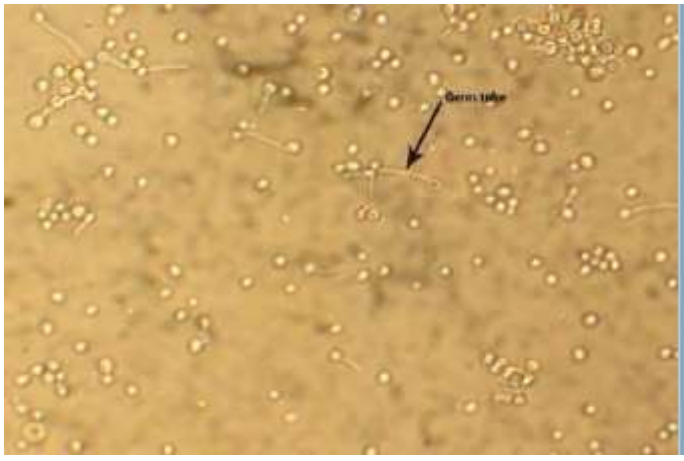

Figure 3. *Germ tube test for candida albicans*

Figure 4. *Coagulase test for S. aureus*

In this study, overall 15.22 percent catheter samples were culture positive. Similar result was obtained in study conducted by Nahirya *et al.*[28] 20.7% of the catheter samples showed positive culture. In a study by Paragioudaki *et al.* [28] high culture positive sample (37.0%) were obtained as compared to our study. In another study, involving 183 catheters Hashemzadeh *et al.*[24] isolated organisms from 9.8% of the sample.

The rate of catheter infection in our study is lower than other studies which may be because of education of healthcare workers about controlling infection, hand washing, skin preparation before catheter insertion, attention to maximal sterile barrier precautions during catheter insertion, appropriate site maintenance, avoiding femoral site and removing unnecessary catheters[29]. The colonization rate was 8.62% and local infection was 6.09%.Whereas, phlebitis was seen at 15.22%. In the study carried out by Morin *et al.*[30] catheter colonization occurred with an incidence of almost 17% and 9% local infection was seen. Similar result was found in a study carried out by Öncü *et al.*[25] where local infection was seen at 5.6%. Our result in case of local infection agreed with the result of these studies, though our study showed lower colonization rate. In contrast study carried out by Parameswaran *et al.*[31] 35.77% local infection which is very high in comparison to our study and this high rate of infection was due to, patients with local catheter infections were not defined into exit site infections. However, a similar result was shown in a study conducted by Nahirya *et al.*[28] where 17.14% had phlebitis. While in other studies phlebitis rate was high in comparison to our study[32].

The rate of Catheter related blood stream infection (CR-BSI) in our study was 0.50% which was far less than a blood stream infection seen in other studies[16, 25, 33].

Age-wise distribution of the cases in our study showed that cases of catheter related infection were higher in the age group of 50-59 and 60-69. Second highest infection were among age group <1 year. But, the correlation between age and CRI was found statistically insignificant (P>0.05). Boni *et al.*[34] also found result comparable to our study.

In present study, Sixteen (53.3%) of the 30 samples were from multiple lumen catheter and the remaining 14 (46.66%) from single lumen catheter. Our result shows lower infection rate than the result of Subba Rao *et al.*[35], where the rate of colonization of peripheral catheter was 52.5% while colonization rate in central venous catheters was 62.5%. In another study by Parameswaran *et al.*[31] , incidence of catheter related infection with the triple lumen was 39.8%. In contrast, a study by Boni *et al.*[34], found 13.6% of central venous catheter colonized while 15% of the peripheral catheters were colonized.

In this study, high infection was found in 13 (43.33%) catheters in place for 2-5 days and 17 (56.66%) catheters in place for 6-11 days. In a study by Subba Rao *et al.*[35], rate of positive tip culture was found in 51.2% catheters in place for 48-96 hours and 60.5% catheters in place for more than 96 hours. In study by Öncü *et al.*[25], the catheter related infection (CRI) rate was higher for CVCs kept in place for ≥ 8 days (24.2%) in comparison to catheters kept in place for ≤ 7 days (12.9%). Another study by Boni *et al.*[34] the positive culture was 33% between 1 to 6 days of hospitalization, 40% between 8 to hospitalization and over 16 days of hospitalization positive culture was 27%. In MICU, CCU, NMW infection rate was 10%, in DBC it was 6.66% and in NSW and CCU it was 3.33%.

The organisms causing infection were Gram-positive cocci 60%, Gram-negative bacteria (GNB) 30% and the Yeasts 10%. The findings of Bouza *et al.*[16], also showed a close result with Gram-positive bacteria comprised 70.7% of all isolates, and Gram-negative bacteria 22.2%. Yeasts were isolated from 7.2% of catheter tip samples. In this study, *S. aureus* as the most common bacterial pathogen isolated from catheters of patients followed by *S. epidermidis* which resembles the study done by various workers[25, 26, 31].

In this study, vancomycin (100%) was found to be the most effective antibiotic against *S. aureus* which was in agreement with other studies[16, 28, 36]. Another effective antibiotic were amoxicillin, gentamicin and ciprofloxacin with a susceptibility of 60 percent. This result was similar with other finding Nahirya *et al.*[28] 60% of the isolated *S. aureus* were resistant to cefoxitin (MRSA). Similar pattern were obtained by other studies[28, 36]. All the *S. epidermidis* isolated in our study were sensitive to vancomycin, which agrees the result by other studies[36, 37]. Gentamicin was active against 42.9% of the isolates and similar result was obtained by Nahirya *et al.*[28]. In our study, all the four *P. aeruginosa* isolates were susceptible to meropenum and 50% susceptible to piperacillin-tazobactam. Parameswaran *et al*, [31] reported that 85.7% isolates were sensitive to piperacillin-tazobactam and 78.6% sensitive to meropenem. One out of two *E. coli* isolates (50%), in our study, was sensitive to gentamicin, 50% with co-trimoxazole and 50% with ciprofloxacin each. Nahirya *et al.*[28], reported 100% of the *E. coli* isolates to be sensitive to gentamicin, 100% to ciprofloxacin and 100% resistance to co-trimoxazole. Among *Acinetobacter* isolates all the two isolates were 50% susceptible to gentamicin, amikacin, meropenem and ciprofloxacin while piperacillin-tazobactam was active against both of the isolates. Only one isolate was susceptible to ceftazidime and one was

intermediate toward it. Contrary to our result Parameswaran *et al*.[31], reported *Acinetobacter baumannii* isolated from a patient with CRBSI was resistant to all routine and reserved drugs.

CONCLUSIONS

The study conducted in Shahid Gangalal National Hospital revealed lower catheter related infection. *S. aureus* was the predominant organism followed by *S. epidermidis* causing infection. *S. aureus* isolated during the study were mostly susceptible towards gentamicin, amoxicillin and ciprofloxacin (60%) and most effective drug was vancomycin. The highest number of cases was observed in the age group of 50- 59 and 60-69. ICU showed higher number of infection as compared to other wards. Similarly, multi lumen catheters were more prone to infection than single lumen catheters and longer duration of catheterization was more associated with infection than shorter duration of catheterization although these variables did not correlate with each other. The association between infection and age, single lumen catheters and multi lumen catheters, longer duration of catheterization and shorter duration of catheterization is statistically insignificant (P>0.05).

ACKNOWLEDGEMENTS

It gives me an immense pleasure to express my profuse gratitude and heartfelt appreciation to my supervisors, Mr. Avishekh Gautam, Lecturer, Department of Microbiology, St. Xavier's College, Maitighar and Dr. Arun Maskey, Consultant Cardiologist, Shahid Gangalal National Hospital, Bansbari, Kathmandu, Nepal for their constant inspiration and encouragement, tremendous support, constructive suggestion and guidance during the entire period of this research work.

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Medically Important *Vibrios* in the Sewage of Kathmandu Valley in Winter Dry Season

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ABSTRACT

Aquatic environment is the natural dwelling place for Vibrio species. With the objective to isolate Vibrios of medical importance (both cholera and non-cholera) from sewage system of Kathmandu Valley, a total of 60 random samples were collected from 10th November 2010 to 6th February 2011 at different sewer sites discharging sewage into the Bagmati River. The samples, Moore's technique based swabs submerged in alkaline peptone water (broth) of pH 8.6, were transported to the research laboratory of Shi-Gan International College of Science and Technology (SICOST) maintaining cold condition (ice-chest). The samples were first enriched at 37^{0}C for 8 hours followed by overnight incubation of culture on thiosulfate-citrate-bile salts-sucrose (TCBS) agar at 37^{0}C. The TCBS plates with colonies resembling V. cholerae and other Vibrios were subjected to identification based on standard bacteriological procedures. Preliminarily identified colonies of V. cholerae were further sub-cultured on nutrient agar (NA) and the colonies from NA were serotyped using polyvalent V. cholerae 01 antisera. Out of the total 57 isolates of Vibrios, V. parahemolyticus (45.6%) dominated the population followed by V. cholerae (21%) and others. Among V. cholerae, hundred percent of V. cholerae serotypes (n=12) were found to be V. cholerae Non-O1 (NAG). All the NAG isolates were subjected to antibiotic susceptibility testing against commonly prescribed antibiotics to treat vibrios related infections in Nepal. Two NAG isolates were found to be multidrug resistant. Presence of both cholera and non-cholera Vibrios in sewage of Kathmandu Valley is a potential indication for possible outbreaks of Vibrios infection. These findings highlights the necessity of regular monitoring and surveillance of sewage system and development of appropriate public health strategies such as maintenance of proper sanitation and safe hygienic practices in order to prevent possible outbreaks of Vibrios in future.

Keywords: *Vibrio cholerae, Moore's technique, sewage, Bagmati River, Nepal*

INTRODUCTION

Aquatic environment is the natural lodgement for *Vibrio* species [1-4]. They exist in association with a number of vertebrate species e.g. fish, zooplanktons and in some instances plants[5]. Of them, many species have acquired increasing importance because of the association of several of its members with human disease. The most feared of *Vibrio* species is *V. cholerae*, the causative agent of cholera, a devastating disease of global significance with high morbidity and mortality[6-7]. Recently, WHO has estimated 3–5 millions cases and 100,000–120,000 deaths due to cholera every year[7]. Other important *Vibrios* of medical importance are *V. parahemolyticus, V. vulnificus, V. mimicus,* and to a lesser extent *V. fluvialis, V. furnissii, V. hollisae, and V. damsela*. Many studies have also implicated *V. alginolyticus* and *V. metschnikovii* in human disease, although their complete significance has not yet been established. The virulence of all medically important *Vibrios* is aided by a variety of traits that help breach human defenses[3].

Bagmati is the biggest river running across the Kathmandu Valley. This river also has religious importance especially for *Hindu* devotees. The river water is also being used to clean the green and leafy vegetables before taking to market. However, during recent years, *Bagmati River* is facing environmental and ecological challenges. Quality of water and river integrity has adversely changed due to anthropogenic activities. It has become an ultimate urban drainage as well as waste dumping site. The river contains large amounts of untreated sewage, and large levels

of water pollution. These statements are supported by the reports of isolation of *V. cholerae* and other medically important *Vibrios* from the sewage of Kathmandu Valley during peak rainy season[8].

In Nepal, outbreak of cholera occurs mainly during rainy season[9-13] and mainly associated with *V. cholerae* O1 biotype El Tor Ogawa[9]. Strains of *V. cholareae* O1 (Hikojima) and *V. cholerae* O139 have also been reported.[9&10] This is true even in the Katmandu Valley where the capital city is located.[9] However, all outbreaks are associated with poor environmental sanitation resulting in contamination of water and foods. Environmental surveillance for medically important *Vibrios* in *Bagmati River* could be potential tool to control *Vibrios* infections. So, this study reports the presence of medically important *Vibrios* (both cholera and non-cholera) in the sewage of *Bagmati River*.

EXPERIMENTAL PARTS

Sample collection

A total of 60 random samples were collected from the different sites during dry winter season (10^{th} November 2010 to 6^{th} February 2011) of sewer system of Kathmandu Valley using principle based on Moore's technique[14]. Briefly, this technique involves the cotton gauge (swab) wrapped on one end of a piece of six inch diameter pipe (five inch long) placed horizontally into the sewerage in opposite to sewerage flow for overnight. Samples [Moore's technique based swabs were submerged in alkaline peptone water (broth) pH of 8.6] were transported to Research Laboratory of SICOST, *Kathmandu* in cold condition (ice chest).

Sample processing

The samples were incubated at 37^0C for 8 hours followed by culture on TCBS agar and incubated at 37^0C for overnight. The TCBS plates showing colonies resembling *V. cholerae* and other *Vibrios* were subjected for identification following standard bacteriological procedures. The organisms were inoculated into triple sugar iron (TSI) agar. The TSI reaction as alkaline or acid slant, acid butt with no gas and H_2S was suspected to be *V. cholerae.* Based on the TSI reaction, suspected colonies were sub-cultured on nutrient agar (NA) and the colonies on NA were subjected for serotyping using polyvalent *V. cholerae* 01 sera as described by Feeley *et. al.* (1974).[15] For the characterization of other species (*V. cholerae* 01 and other medically important *Vibrios*), colonial characters and biochemical tests (such as sucrose fermentation, lysine utilization, motility, indole test, oxidase, swarming, growth on 0, 2, 3, 6% NaCl and others) were employed.

Antibiotic susceptibility test

The *V. cholerae* isolates were subjected to *in-vitro* susceptibility test employing Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute and result was interpreted based on the provided guidelines [16].

RESULTS AND DISCUSSION

In this study, most of the medically important *Vibrios* were isolated with the dominance of *V. parahaemolyticus* (see Table 1). Rai *et al*., 2008 has reported the presence medically important *Vibrios* in the sewage of Kathmandu Valley with the dominance of *V. cholerae* during peak rainy season[8]. Dissimilarity in the distribution of *Vibrios* might be attributed to the seasonal variation and survival of *Vibrios* in the sewage system.

Table 1**:** *Distribution of medically important Vibrios in sewage samples*

Vibrio Species	**Number**	**%**
V. parahaemolyticus	26	45.6
V. cholera	12	21.0
V. alginolyticus	11	19.3
V. furnissi	4	7.0
V. fluvialis	2	3.5
V. vulnificus	2	3.5
Total	**57**	**100**

V. cholerae was isolated at the rate of 21% (12/57) which is lower than other studies reported elsewhere. In Argentina, Bangladesh, Japan and India, the isolation rate ranged from 60% to 100% [17- 20]. In this study, samples were collected during winter dry season. Variation in isolation rate might be associated with isolation techniques,

seasonal variation and other physio-chemical parameters of sewage. Further research will explore the distribution of *Vibrios* including *V. cholerae* in sewage with all attributes.

In Nepal, Outbreak of cholera is mainly due to *V. cholerae* 01 El Tor Ogawa [9]. However, strains of *V. cholerae* 01 Hikojima and Inaba are also reported from many outbreak cases [9, 10]. Also, *V. cholerae* 0139 had also been reported in few cases[9]. Outbreaks of cholera occur each year with the beginning of summer/rainy season (continues to post rainy season)[10-13]. In this study, we collected samples during winter dry season where cholera outbreaks sparsely. It is interesting that this study was unable to detect the *V. cholerae* O1 using poly O1 anti-sera during winter season. Therefore, this finding correlates the presence/ absence of cholera *Vibrios* in sewage of Kathmandu Valley with cholera outbreaks. Rai *et al* (2008) reported the presence of cholera *Vibrios* in the sewage of Kathmandu Valley during rainy season[8]. In addition to this, epidemiologically, all biochemically resembling cholera *Vibrios* are important for possible cholera outbreak because according to the Finkestein (1973), *Vibrios* resembling *V. cholerae* but failing to agglutination in cholera antisera had been strongly implicated as causative agents of both sporadically occurring and focal cholera like diarrheal disease [21]. *Vibrios* infection other than cholera has not been reported in Nepal though many *Vibrio* species are responsible for human pathogenesis [3].

***Table 2**: Antibiogram of V. cholerae isolates*

Number	Antibiotics	Sensitive isolates		Resistance isolates	
		Number	% sensitive	Number	% resistance
12	Amikacin	12	100	----	0
	Nalidixic acid	10	83.3	2	16.7
	Norfloxacin	12	100	----	0
	Cloramphenicol	10	83.3	2	16.7
	Cefexime	11	91.7	1	8.3
	Tetracycline	10	83.3	2	16.7
	Ofloxacin	12	100	----	0
	Polymyxin B	9	75	3	25

All biochemically resembling cholera *Vibrios* (n=12) Viz. sucrose fermenter, oxidase positive, indole positive, lysine utilizer which grow on 6% NaCl containing nutrient broth and others, were subjected to antibiotic susceptibility test (see Table 2) where only two isolates were found to be multidrug resistant (resistant to tetracycline, nalidixic acid and chloramphenicol). Emergence of drug resistance strain of *V. cholerae*[22-25] is global concern which could be addressed by public health strategies, especially by means of environmental surveillance. Presence of multidrug resistant cholera *Vibrio* in the sewage Kathmandu Valley, may aid to develop specific public health strategy for the intervention of possible outbreak.

CONCLUSION

Presence of medically important *Vibrios* in the sewage of Kathmandu Valley during winter dry season is potential threat for possible future outbreak. Sewage surveillance may aid to predict possible outbreak as well as to develop public health strategies and, therefore, demands proper sanitation, consumption of safe drinking water and practice of personal hygiene to prevent outbreak in future.

ACKNOWLEDGEMENT

The authors acknowledge Prof. Dr. Shiba Kumar Rai for his suggestions and encouragements and entire team of SICOST.

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Proceedings of First International Conference on Infectious Diseases & Nanomedicine-2012
(December 15-18, 2012, Kathmandu, Nepal)

Recent Status of Major Infectious Diseases in Nepal

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ABSTRACT

Given the complexity of the occurrence and distribution of the communicable diseases, an attempt has been made to present the major infectious diseases in Nepal. This paper provides an overview of the current burden of infectious disease in Nepal and also highlights current focus of disease prevention and future directions that might enable national and global policy makers to deal more effectively with these communicable diseases.

Keywords: *infectious disease, mass immunization, epidemic,*

INTRODUCTION

Nepal, a Himalayan South Asian country, is enriched with a diverse cultural, climatic and geographical variation. The diverse climatic conditions of the country accompanied with poor hygienic practices and sanitation is offering home for the continuous emergence and reemergence of several life threatening human infectious diseases[1,2].This issue has been closely tied to the low income rate of the people [3]. Despite the rapidly increased rate of urbanization from 14% in 2001 to 17% in 2011, Nepal is predominantly a rural country. As majority of the population lives in rural areas with very limited health care facilities, the diseases burden is much higher in rural settings compared to the urban areas [1-3].

Although the pattern of diseases might change constantly, communicable diseases remain the leading cause of mortality and morbidity in the country. Some diseases (for example, cholera, acute gastroenteritis) are endemic to all regions of the country while other disease such as, kala-azar are particular to certain geographical regions. Additionally, increased incidence of emerging diseases such as influenza mutants, dengue, Japanese encephalitis, chikungunya, and leptospirosis in the recent years is further compounding the situation as there is high potentiality of outbreaks with widespread morbidity and mortality at any time[4,5].

Some diseases such as mumps, measles are reported with lower numbers due to active mass immunization campaigns. However, diseases such as H5N1 influenza, Japanese encephalitis, malaria, and leptospirosis are difficult to identify without specialized laboratory testing which is often not available throughout all regions of the
country. In many rural settings, cases of some diseases are often identified only through their clinical signs and symptoms[4,6].

CURRENT BURDEN OF MAJOR INFECTIOUS DISEASES IN NEPAL

Malaria

Malaria is a major public health problem in Nepal. Sixty-five districts out of seventy five are at risk for malaria, 13 of which are highly endemic where more than 70% of malaria cases originate. Government of Nepal, Department of Health Services reported that among the 133,730 cases diagnosed as probable malaria (clinical cases) only 2,857 were laboratory- confirmed [6]. Further, the number of laboratory confirmed malaria cases has decreased from 3,241 in 2010-2011 to 2,857 in 2011-2012 [6,7]. However, the proportion of *P. falciparum* cases has substantially increased from 15.7 % to 46 % during those years [6,7]. The Annual Parasite Incidence (API) rate per 1000 has been decreased from 0.16 to 0.11 in 2011/2012.There is a decrease in Slide Positivity Rate (SPR) compared to the previous year [6].

A review of external experts conduced in 2010 commended the country for its achievements and recommended to move forward for pre-elimination consolidating the gains achieved so far, sustaining the downward trend in malaria

morbidity/mortality and maintaining the outbreak free status. Recently, interventions are being focused on early diagnosis and prompt treatment, prevention of transmission through selective vector control and training of programme staff in case diagnosis, management and outbreak response, by collaboration among Governmental and non-governmental organizations [6-9].

Tuberculosis

Tuberculosis (TB) is one of the major public health problems in Nepal. According to Department of Health Service and National Tuberculosis Center Nepal, about 45 percent of the total population is infected with TB, of which 60 percent are adult [6]. Every year, 45, 000 people develop active TB, out of them 20,580 have infectious pulmonary disease. Treatment by Directly Observed Treatment Short course (DOTS) has reduced the number of deaths; however 5,000-7,000 people are still dying per year by TB. Expansion of this cost effective and highly successful treatment strategy has proven its efficacy in reducing the mortality and morbidity in Nepal. Among the 36,764 total TB cases registered during 2011/2012, 15,059 were new sputum smear positive cases, with the case detection rate of 73% and the treatment success rate of 90 % [6,10]. A Drug Resistance Survey conducted in 2011 showed multidrug resistance (MDR) at 2.2% among new TB cases and 15.4% among previously treated cases [11].

WHO estimates prevalence for all types of tuberculosis cases for Nepal at 74,000 (243 per 100,000 population) [11] while National Tuberculosis Centre, Nepal estimates that the all forms of cases is at 50, 000 (163 per 100,000 population) [10]. By achieving the global targets of diagnosing 70 % of new infectious cases and curing 85 % of these patients will prevent 30,000 deaths over the next five years. High cure rates and sputum conversion rate will reduce the transmission of TB and lead to a decline in the incidence of this disease, which will ultimately help to achieve the goal and objectives of TB control [6].

HIV/AIDS

Since its first reported case of AIDS in 1988, the HIV epidemic in Nepal has evolved from a "low-prevalence" to a "concentrated" epidemic [12]. As of 2011, Department of Health Service of Nepal reported that 50,288 adults and children are infected with the HIV virus in Nepal which is estimated to be 0.3 % of the total adult population of the country. Among the total infections, 7.6% are children under 14 years while 6.5% are people over 50 years. More than two-thirds of the infection is among males. Among women, about 84% are in the reproductive age group of 15-49 years [1,6,7]. However, in overall HIV prevalence among adults (aged 15–49 years) is gradually declining owing to the interventions targeting to these key populations [4, 12]. The estimated number of annual AIDS deaths of all ages is projected to decrease from 4,722 (2011 estimate) to 1,576 in 2015. This decline is most likely due to the increase of the number of people on antiretroviral treatment. The treatment needs were estimated (using CD4 count <350) at 27, 288 (adults: 25,169, children: 2,119) in 2011. These numbers are projected to rise to 28,791 (adults: 26,896, children: 1,896) in 2015 [6].

Lymphatic filariasis

Lymphatic Filariasis (LF) is also a public health problem in Nepal. The disease has been reported from a wide range of topographical variation- from 300 feet of altitude above the sea level to 5,800 feet mountainous regions, primarily affecting the poorer community of rural and slum areas. However, studies have shown that more cases have been reported in Terai as compared to the hills [6]. Although endemic in 60 out of 75 districts, elimination by 2020 is in progress with the use of mass drug administration (MDA) [4]. *Wuchereria bancrofti* is the only recorded parasite in Nepal with its mosquito vector -*Culex quinquefasciatus* [6].

Visceral leishmaniasis

In Nepal, visceral leishmaniasis (VL), also called Kala-azar, is mainly confined to the southern plains of central and eastern regions bordering to the VL endemic districts of Bihar, India. It is known to be endemic in southern Terai since long time as reported by an Indian scientist Raghavan in 1953 [1, 6,7,13]. However, a few sporadic cases are occasionally recorded from other parts of the country. Principle vector incriminated is the sand fly, *Phlebotomus argentipes.* In the endemic areas, children and young adults are its principal victims. Currently Kala-azar is endemic in twelve districts [6]. The incidence of Kala Azar is 1.33/10 000 with a case–fatality rate (CFR) of 0.59% [4]. Kala-azar and HIV/TB co-infections have emerged as a health problem in recent years [6].

Dengue fever

Dengue, a mosquito-borne disease has been reported in Nepal in the form of Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) as early as 2006 [1, 6]. *Aedes aegipti* is reported as the mosquito-vector. Epidemiology and Disease Control Division/ National Public Health Laboratory of Nepal reported all 4 sub-types (DEN-1, DEN-2, DEN-3 and DEN-4) of Dengue virus in Nepal [6, 7, 13].

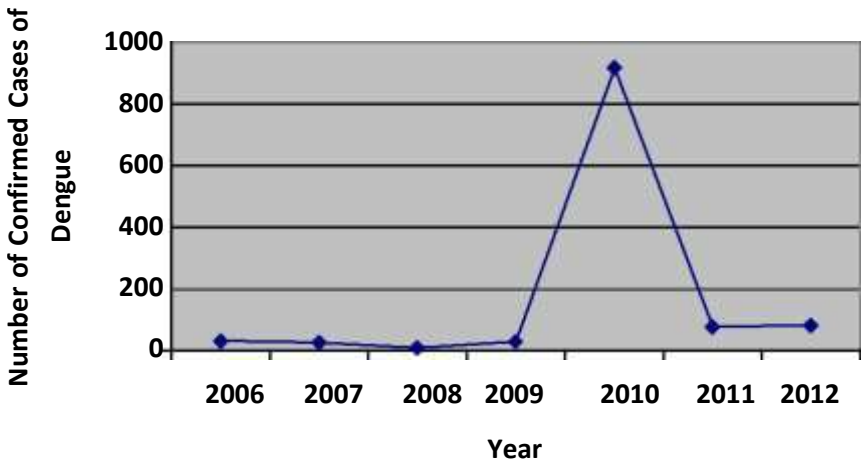

Figure-1 Incidence of Dengue in Nepal from 2006-2012 [1,6,7,13]

As mentioned in Figure-1, major dengue has occurred in 2010 with 917 reported cases as compared to 32 confirmed dengue cases reported in 2006 [6, 7, 13]. During 2011, 79 confirmed cases were reported from 15 districts and a total of 82 confirmed cases were reported by 2012 [6].

Japanese Encephalitis

Culex tritaeniorhyncus is the principal vector of JE in Nepal. Total of 26,667 cases and 5,381 deaths have been reported with average case fatality rate of 20.2% in an aggregate since 1978 -2005 [14]. In 2008–2009 a total of 1355 cases of acute encephalitis syndrome (AES) were reported from 60 districts, of which 119 were confirmed cases of Japanese encephalitis (JE) [13]. In 2011, the number of reported cases of JE is 126 [15].

Leprosy

During 2008–2009, 4565 new cases of leprosy were reported and put under multidrug therapy [13]. In 2010, Nepal was successful in eliminating leprosy as a public health problem. WHO-world health statistics showed that the number of reported cases of leprosy in Nepal as 3184 in 2011 [15]. However, the Government of Nepal, Department of Health Service annual report- 2011/2012 states that by the end of the Fiscal year 2011/12 there were 2430 leprosy cases receiving MDT in the country, which makes the registered prevalence rate of 0.85/10,000 populations at national level i.e. below the cut-off point of <1 /10,000 population set by WHO to measure the elimination of leprosy as public health problem [6].

Epidemic prone diseases

Cholera and gastroenteritis are endemic in all areas of the country. The incidence of diarrhoea per 1000 children is increased from 378 in 2007/2008 to 598 in 2009/2010, although case–fatality rates in children decreased from 0.15 in 2007/2008 to 0.00 in 2009/2010 [1,13]. Number of reported cases in 2011 are 12 [15,16]. Although none of the human cases of mutant influenza (H1N1 and H5N1) were detected in humans in Nepal to date, continuous outbreaks in poultry has increased the risk of human transmission [6].

Vaccine preventable diseases

Nepal was polio free in 2011. Case-based surveillance for measles is functional throughout the country. In 2011, there were 2359 reported cases of measles [4]. Nepal has targeted measles elimination by 2016. In 2011, the total number of cases recorded for mumps and rubella were 39023 and 1175 respectively [15]. Although Nepal has achieved the maternal/neonatal tetanus elimination status in 2005, there are 95 reported case of neonatal tetanus in 2011 with the total of 193 [4,6, 15].

MAJOR CHALLENGES OF DISEASE CONTROL

Continued attention on environmental determinants for better health, such as safe water, sanitation, hygiene and health-care waste management, remains a huge challenge for the control of communicable diseases. Health care

waste management particularly poses a constant threat to the public health system. Nepal is also prone to many natural disasters, particularly earthquakes, floods and landslides. Effort and investment are needed to prepare for and mitigate the impact of natural disasters and climate change.

Despite the ongoing efforts for surveillance and response to prevent and control communicable diseases, Nepal is still facing challenges in accurately identifying, diagnosing and reporting infectious diseases owing to the remoteness of communities, urban-centered healthcare infrastructures, and lack of specialized laboratories with skilled professionals.

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