DOI: 10.7860/JCDR/2013/5193.2955

Application of WHONET in the Antimicrobial Resistance Surveillance of Uropathogens: A First User Experience from Nepal

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ABSTRACT

Introduction: WHONET is a freely downloadable, Windows-based database software which is used for the management and analysis of microbiology data, with a special focus on the analysis of antimicrobial susceptibility test results. Urinary Tract Infections (UTI) are a common medical problem and they are responsible for notable morbidity among young and sexually active women.

Objectives: The major objective of this study was the utilization and application of the WHONET program for the Antimicrobial Resistance (AMR) surveillance of uropathogens.

Methods: A total of 3209 urine samples were collected from patients who visited Manipal Teaching Hospital with a clinical suspicion of UTI, during December 2010 to July 2011. The isolation and characterization of the isolates were done by conventional methods. Antimicrobial Susceptibility Testing (AST) was performed by Kirby Bauer's disc diffusion method. The data entry and analysis were done by using the WHONET 5.6 software.

Results: Out of the 3209 specimens, 497 bacterial isolates were obtained and they were subjected to AST. *Escherichia coli* (66.2%) was the commonest bacterial isolate, followed by *Enterococcus* species (9.3%), *Staphylococcus aureus* (5.0%), and *Klebsiella pneumoniae* (4.2%). Among the gram-negative enteric bacilli, a high prevalence of resistance was observed against ampicillin and ciprofloxacin. The gram negative nonfermenters exhibited a high degree of resistance to ceftazidime. *Staphylococcus* species. showed a moderately high resistance to co-trimoxazole. One isolate was Vancomycin Resistant *Enterococci* (VRE).

Conclusion: This study, a first of its kind which was done in Nepal, was carried out by using the WHONET software to monitor, analyze and share the antimicrobial susceptibility data at various levels. This study was also aimed at building a surveillance network in Nepal, with the National Public Health Laboratory, Nepal, acting as a nodal centre. This would help in the formulation of antibiotic policies and in identifying hospital and community outbreaks at the nodal centre, as well as in sharing information with the clinicians at the local level.

Key Words: WHONET, Antimicrobial resistance surveillance, Urinary tract infection

INTRODUCTION

The World Health Organization has established a program to tackle the problem of antimicrobial resistance. It is known as the Antimicrobial Resistance Monitoring (ARM) program. It requires accurate and easily accessible data on antimicrobial resistance to support the decision making and to take action from the local to the global level. To achieve all this, the WHO has devised an electronic format which is named as WHONET [1]. WHONET is a freely downloadable, Windows-based database software which was developed for the management and analysis of microbiology data, with a special focus on the analysis of the antimicrobial susceptibility test results. It is also possible to retrieve, correct and print clinical records by using this program.This software has been developed since 1989 by the WHO Collaborating Centre for the surveillance of Antimicrobial Resistance, which is based at the Brigham and Women's Hospital in Boston. This software is used by clinical, public health, veterinary, and food laboratories in over 90 countries, to support the local and national surveillance programs. The WHONET analytical tools facilitate the understanding of the local epidemiology of microbial populations; the selection of antimicrobial agents; the identification of hospital and community outbreaks; and the recognition of quality assurance problems in laboratory testing.

Unfortunately, WHONET has not been used for antimicrobial susceptibility data analysis in Nepal so far. The Department of Microbiology, Manipal Teaching Hospital, the affiliated teaching hospital of Manipal College of Medical Sciences, Pokhara, Nepal, is the first institute to employ the WHONET 5.6 program to collect, collate and analyze the antimicrobial susceptibility data of all clinical isolates since December 2010.

It is an established fact that Urinary Tract Infections [UTI] remain one of the most common bacterial infections and the second most common infectious condition in the community practice [2]. About 150 million people are diagnosed with UTI each year [3]. In the present scenario, the antimicrobial drug resistance amongst the major uropathogens has posed a global threat [4]. The treatment becomes even more challenging in the presence of risk factors such as the extremes of age, co-morbidities like Diabetes mellitus, hepatic failure, renal failure and immunosuppression. Various studies which have been done worldwide, have shown changing patterns in the aetiology of UTI [5,6]. The review of literature has not revealed many studies from Nepal which have pertained to UTI and the antibiotic resistance of pathogens [4,7-9]. The knowledge about the prevalent trends of the uropathogens and their susceptibilities to various antibiotics, are essential to formulate guidelines for the empirical treatment of UTI, while awaiting the culture and sensitivity reports.

Realizing the high prevalence of drug resistance in the bacterial population which causes UTI, the present study was carried out by the application of the WHONET 5.6 software, with the aim of the antimicrobial resistance surveillance of the uropathogens in our hospital.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology, Manipal Teaching Hospital, Pokhara, Nepal, from December 2010 to July 2011. A total of 3209 urine samples which were received in the laboratory for assessing the culture and sensitivity of aerobic bacteria, were subjected to this study. The isolation of pathogenic bacteria from urine specimens and their identification to the species level, were performed by standard methods [10]. The antimicrobial sensitivity was tested by the Kirby-Bauer disc diffusion method which was standardized as per the Clinical Laboratory Standards Institute (CLSI) guidelines [11]. The antibiotics were selected according to the WHO model list of essential drugs. For the internal quality control, the *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and the *Ps. aeruginosa* (ATCC 27853) strains were used [1].

The main requirement for the compilation of the data was a PC in which the WHONET 5.6 software was installed [12]. Customization of the software was carried out by laboratory configuration. The antimicrobials which were routinely tested in the laboratory, the patient care areas which were served and other data which pertained to the patients and the specimens were configured. All the data entry was done manually on a daily basis.

RESULTS

A majority of the patients 2727 (85.0%) were adults. Out of the 3209 urine samples, 2772 (86.4%) were from indoor patients and 437 (13.6%) were from the outpatients department. From these samples, 497 bacterial isolates were obtained. The gender distribution showed that more organisms were isolated from females (68.2 %) than from males (31.8 %). The spectrum of the urinary tract pathogens which were identified, is shown in [Table/Fig-1]. *Escherichia coli* was the commonest bacterial isolate (66.2 %).

The antibiograms of the different isolates are depicted in [Table/Fig-2, 3 and 4]. Amongst the gram negative bacilli, 84.7% *E coli* were resistant to ampicillin, while amikacin resistance was seen in only 4.4% *E coli*. 76.2% nonfermenters were resistant to ceftazidime, but no resistance was seen against imipenem [Table/Fig-4]. 57.1% *Staphylococci* were resistant to the trimethoprim+sulfa combination, but interestingly, all were sensitive to gentamicin and vancomycin.

DISCUSSION

A routine surveillance is essential to monitor and control the spread of antimicrobial resistance. The antibiotic susceptibility test results are stored in paper files or in computer files in a large number of laboratories around the world, especially in the developing countries, which usually makes them inaccessible. Most of the times, there is no uniformity in the storage of the data, thus making it impossible for analysis and comparison. WHONET is an information system which was developed to support the World Health Organization's (WHO's) goal of the global surveillance on the bacterial resistance to antimicrobial agents [13]. The data can be shared with another laboratory which works on the WHONET program for further collaboration and the implementation of local, national or global surveillance networks.

Uropathogens	No. of isolates (%)
Escherichia coli	329 (66.2)
Enterococcus species	46 (9.3)
Staphylococcus aureus	25 (5.0)
Klebsiella pneumoniae	21 (4.2)
Acinetobacter species	15 (3.0)
Coagulase negative *Staphylococcus*	15 (3.0)
Other Gram negative bacilli*	36 (7.2)
Candida species	10 (2.0)

[Table/Fig-1]: Spectrum of uropathogens isolated (n=497)
*Other Gram negative bacilli include *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas* spp., *Proteus vulgaris*, and *Klebsiella* spp. (other than *Klebsiella pneumoniae*)

Antibiotic	%R (95%C.I.)
Ampicillin	84.7 (80.5, 88.1)
Cefazolin	58.9 (53.4, 64.2)
Amikacin	04.4 (1.8, 9.8)
Gentamicin	31.9 (27.3, 36.9)
Ciprofloxacin	60.5 (53.7, 66.9)
Norfloxacin	58.1 (52.9, 63.1)
Trimethoprim+Sulfamethoxazole	58.8 (53.6, 63.8)
Nitrofurantoin	18.1 (14.3, 22.6)

[Table/Fig-2]: Antibiotic resistance amongst *Enterobacteriaceae* (*n* = 377)

Antibiotic name	%R (95%C.I.)
Oxacillin	34.4 (19.2, 53.2)
Gentamicin	00.0 (0.0, 37.1)
Ciprofloxacin	36.7 (20.6, 56.1)
Norfloxacin	20.0 (3.5, 55.8)
Trimethoprim+Sulfamethoxazole	57.1 (39.5, 73.2)
Vancomycin	00.0 (0.0, 43.9)

[Table/Fig-3]: Antibiotic resistance amongst *Staphylococcus* species. (*n* = 40)

Antibiotic	%R (95%C.I.)
Piperacillin	39.1 (20.4, 61.2)
Sulbactam	16.7 (0.9, 63.5)
Ceftazidime	76.2 (52.5, 90.9)
Imipenem	00.0 (0.0, 43.9)
Amikacin	30.4 (14.0, 53.0)
Gentamicin	47.6 (26.4, 69.6)
Tobramycin	37.5 (10.2, 74.1)
Ciprofloxacin	31.8 (14.7, 54.9)
Norfloxacin	33.3 (1.8, 87.5)

[Table/Fig-4]: Antibiotic resistance amongst Gram negative non-fermenters (n = 25)

The scientific literature on antimicrobial resistance, which has been published by utilizing the WHONET software in the developing countries, is limited. One study was conducted in 11 hospitals of Beijing in 1995 [14]. A National Electronic Network was launched in Greece on an earlier version of the WHONET software *version* 4 and the study was conducted in 19 hospitals of Greece. Their experience of four years helped them in identifying the main factors which were responsible for the emergence of antimicrobial resistance. It also helped in identifying the subpopulations of resistant bacteria for molecular studies and it facilitated the formation of a hospital based, empirical therapy [15]. Manninen et al., recommended the use of the WHONET software to get laboratory specific breakpoints to avoid false reporting of the resistance [16]. Sharma *et al* conducted a surveillance on the antimicrobial resistance by the application of WHONET and advocated its use for forming a hospital drug policy, the identification of hospital outbreaks and for the recognition of quality control problems in the laboratory [17]. Mochizuki et al., analyzed the hospital laboratory microbiological data by using WHONET 5 to acquire information about the antimicrobial resistance of the *Staphylococcus aureus* strains among every ward. They recognized WHONET 5 as an analysis and surveillance tool which could be used by every infection control team to survey the suspicious wards [18]. Stelling et al., evaluated WHONET and a space-time permutation scan statistic for a semi-automated disease outbreak detection in collaboration with WHONET-Argentina, for the detection of local and regional outbreaks of *Shigella* species. They concluded that WHONET based, disease surveillance incorporating, statistical cluster detection methods can enhance the infectious disease outbreak detection and response [19].

In Nepal, we are the first Microbiology laboratory to utilize the WHONET 5.6 program to collect, collate and analyze the anti-microbial susceptibility data of all clinical isolates since December 2010. To establish an AMR surveillance network, the National Public Health Laboratory (NPHL), Nepal, felt the need of a national workshop on the laboratory based surveillance of antimicrobial resistance and the application of WHONET. These workshops, in collaboration with the World Health Organization, Country Office, Nepal, were organized at the Department of Microbiology, Manipal College of Medical Sciences, Pokhara, Nepal. It imparted a hands-on training to the personnel who were working in various government laboratories in Nepal.

The WHONET program has a user-friendly interface which permits many types of analysis. The options include isolate line-listings and summaries such as organism frequencies over time, antimicrobial susceptibility test statistics, zone diameters and MIC histograms, antibiotic scatter-plots and regression curves, and antibiotic resistance profile line-listings and summaries. It also has a number of alert features which permit the detection of unlikely or important results, as well as the possible hospital or community outbreaks of bacterial or non-bacterial species.

During the data entry, an alert would appear when we had entered an uncommon resistance pattern like vancomycin resistant *Enterococci.* The alert also cautioned us about the possible laboratory errors during the entry of the vancomycin resistant *Enterococci.* After getting such alerts, we started reconfirmation of the isolates and the antibiotic resistance pattern. A display on the priority of different antibiotics towards various microbial agents was helpful in the selection of specific antibiotics for different bacterial isolates.

Monthly isolate line-listings and summaries, along with antimicrobial susceptibility test statistics of clinical specimens from different areas of the hospital, were disseminated to the clinicians on a regular basis. Isolate line listing in our hospital during the month of Jan 2011 showed a total of 45 *Escherichia coli,* 4 *Klebsiella pneumoniae* and 9 *Enterococcus* species, along with other bacterial agents*.* The antibiotic scatter-plot analysis helped us in comparing the efficacy of two antibiotics against each other, based on the prevalent antimicrobial susceptibility patterns of uropathogens, for making an informed decision about its suitability. The antibiotic scatter-plot of *Escherichia coli* during the month of Jan 2011, showed that amikacin and nitrofurantoin were the most effective against it and that ampicillin was the least effective.

A large number of studies which have been done on urinary tract pathogens are available in the scientific literature. In the present study, utilization of the WHONET program was done to explore the feasibility of enhancing the local use of the laboratory data and to promote national and international collaborations through the exchange of data.

Analysis of the data which pertained to uropathogens, by the WHONET software, revealed that a majority of the pathogens which were isolated were from adult patients (85.0%), principally from women (68.2%). It has been extensively reported that adult women have a higher prevalence of UTI than men, which principally owes to anatomic and physical factors [20, 21]. *Escherichia coli* (66.2%) was the commonest bacterial isolate, followed by *Enterococcus* species (9.3%)*, Staphylococcus aureus* (5.0%)*,* and *Klebsiella pneumoniae* (4.2%). The antimicrobial susceptibility profiles of gram-negative enteric bacilli revealed a high prevalence of resistance against ampicillin (84.7%) and ciprofloxacin (60.5%). The gram-negative nonfermenters showed a high degree of resistance to ceftazidime (76.2%). *Staphylococcus* species*.* exhibited a moderately high resistance to co-trimoxazole (57.1%). One vancomycin resistant *Enterococcus* isolate was found.

The findings of this study are being disseminated amongst the clinicians in our hospital. This will help them in making an informed choice for the empirical treatment of UTI. This data is being utilized for formulating the hospital antibiotic policy as well. We plan to continue to share such data periodically with all stake holders, to help and guide them in the proper use of antimicrobials.

CONCLUSION

Application of the WHONET 5.6 software provides a uniform and a standardized platform for the management and analysis of microbiology data, with a special focus on the analysis of antimicrobial susceptibility test results. It should be used by the laboratory to collect, collate, analyze and share the data at various levels – local, regional and national. This will enable in building a strong antimicrobial resistance surveillance network in Nepal, with the National Public Health Laboratory, Nepal, acting as a nodal centre. Because of the regional and demographic differences in the susceptibility patterns of pathogens, the AMR surveillance at a nodal centre will guide in the formulation of antibiotic policies and in taking appropriate measures in the identification and control of hospital and community outbreaks.

We hope that other centres in Nepal will also use WHONET and share their data, thereby taking the first step in formulating effective antibiotic policies at local, nodal, regional and national levels.

ACKNOWLEDGEMENTS

We would like to thank all the staff of the Microbiology Department, Manipal Teaching Hospital, Pokhara, Nepal, for their co-operation.

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FINANCIAL OR OTHER COMPETING INTERESTS:
None.

Date of Submission: Oct 18, 2012
Date of Peer Review: Nov 24, 2012
Date of Acceptance: Mar 07, 2013
Date of Publishing: May 01, 2013