o

WHONET Training Course

Module 4 – Introduction to data analysis

Exercises

Brigham and Women’s Hospital

WHO Collaborating Centre for

Surveillance of Antimicrobial Resistance

Boston, Massachusetts, United States

May 2024

**WHONET – Getting Started - Exercises**

This tutorial will illustrate the main features of the user interface for the WHONET data analysis module including the selection of required and optional analysis parameters and the definition of isolate selection criteria. More detailed discussions of the analysis and interpretation of results will be covered in subsequent exercises.

1. **Entering the data analysis screen.**
	1. Open WHONET and select a laboratory.
* Double-click on the WHONET icon on your desktop to begin the software.
* Select “WHONET Test Hospital” and click on “Open laboratory”. This will bring you to the main menu.
	1. Open the “Data analysis” screen.
* On the main menu, click on “Data analysis”. You will see two options: “Data analysis” and “Quick analysis”. For these exercises, select “Data analysis”.
* You will now see the main WHONET analysis screen from which you can select the details for your analyses.
1. **Setting up an analysis: %RIS and test measurements**

In the main analysis screen, there are three sections on the left that you must answer: Analysis type, Organisms, and Data files. On the right, there are some additional options that may be useful to you.

* 1. Analysis type: Click on “Analysis type”. You will see several analysis options. For this first analysis, select the option “%RIS and test measurements”. Click “OK” to return to the main analysis screen.
	2. Organisms: Click on “Organisms”. On this screen, you will see the options available to you on the left side of the screen. On the right side of the screen, you will put your selections.
* From the left of the screen, select *Staphylococcus aureus* (with code “sau”) and *Escherichia coli* (“eco”). You can select an organism in several equivalent ways: 1) double-click on the organism name; 2) single-click on the organism name and then click on the the right-arrow button “–>”; or 3) type the three-letter code into the text box and hit <Enter>. After you select the two organisms, you should see them appear on the right side of the screen.
* There are some other useful options on this screen as well. We will not utilize these features in this exercise, but you can explore them.
	+ “Extended list”: By default, WHONET displays a relatively short list of the most common microorganisms. Click on “Extended list” to see the complete list.
	+ “Search”: You can find organisms of interest by using the “Search” box. You can enter part of the genus name and part of the species name, such as “kleb pneu”, and WHONET will find organisms that match this pattern.
	+ “Organism groups”: Click on “Organism groups”. You will see that WHONET permits you to analyze groups of microorganisms such as “All organisms”, “All Enterobacteriaceae”, “All Salmonella”, or “All fungi”.
	+ “Analyze as one organism”: WHONET generally will analyze each organism selected separately. If you would like WHONET to average results together from multiple organisms (for example “*Klebsiella pneumoniae*”, “*Klebsiella oxytoca*” and “*Klebsiella* sp.”), then click on the option “Analyze as one organism”.
* After you select the two organisms, click “OK” to return to the main analysis screen.
	1. Data files: Use this screen to choose the data file or data files that you wish to analyze.
		+ Click on “Data files”
		+ For this exercise, select the file WHO-TST-2000-01.sqlite. This file includes one month of microbiology laboratory data from a human health hospital. You can select the file: 1) by double-clicking on the file; or 2) by single-clicking on the file and clicking on the right-arrow button “–>”.
		+ After selecting the file, click on “OK” to return to the main analysis screen.
1. **Running the analysis and interpreting the results**
	1. Now that you have given WHONET the details of the analysis to perform, you can proceed with the analysis.
* Click on “Begin analysis”. The analysis will run quickly and display the results to you.
	1. Reviewing the results in the table. You will see a table with the antimicrobial susceptibility test statistics for each of the antimicrobials tested.
		+ In the top-left corner, you will see that there are 86 isolates of *Staphylococcus aureus*.
		+ The output table includes columns such as “Antibiotic name”, “Number”, “Breakpoints”, “%R”, “%I”, and “%S”. If you enter MIC results, then you will also see MIC statistics, such as “MIC50”, “MIC90”, and “MIC Range”.
		+ The antibiotic used to define MRSA is cefoxitin, and you will notice that there are 86 results for cefoxitin with a %R of 11.6%.
		+ If the data file includes disk diffusion zone diameters or MIC values, you will also see the distribution of results by these measurements.
		+ Click on the column “Show hidden columns”. This will show you other fields such as “Antibiotic class” and “Antibiotic subclass” that may be of interest for some.
	2. Review the results in the graph. The lower part of the screen some the same data presented in the table, but in graphical format. You may change the graph by selecting a particular “row” or “column” to be displayed.
* By default, WHONET shows you the graph of percent of isolates resistant to the antibiotics shown.
* In the lower-right, click on the “Row” labeled “Number tested”. This graph is particularly useful: 1) to know which antibiotics were tested by lab (and whether they are appropriate or inappropriate agents for testing); and 2) how frequently each agent is tested, which may suggest issues such as stock outages and selective testing (e.g. “first-line” versus “second-line” testing or “urine” versus “non-urine” panels).
* Click on some of the antimicrobials listed in the lower panel, for example cefoxitin and erythromycin. WHONET will display the zone diameter or MIC distribution of the selected drug. The red lines in the graph represent the interpretative breakpoints. For disk diffusion results, susceptible bacteria will appear to the right of the red lines; resistant isolates are to the left; and intermediate results are between the two lines. For MIC data, the susceptible bacteria will appear to the left and resistant bacteria to the right.
	+ You will only see these zone diameter and MIC histograms if your database includes these measurements.
	+ In the case of the erythromycin distribution, you will see two groups of “resistant” bacteria to the right: “highly resistant” bacteria to the far left and “moderately resistant” bacteria between 9mm and 12mm. The different degrees of resistance would suggest different resistance mechanisms and distinct microbial clones, which could be investigated and confirmed by molecular typing.
* Click on “Continue” to advance from the results for *Staphylococcus aureus* to the results for *Escherichia coli*.
1. **Transferring WHONET results to Excel and other softwares**

The WHONET results that you see on the screen can easily be transferred to other softwares such as Microsoft Excel, Word, or PowerPoint. You can do this either by using “Copy” and “Paste” or by saving the results as a file.

* 1. Copy and Paste
* Click on “Copy table”.
* Now open Microsoft Excel, for example by clicking on your Windows “Start”, “All Programs” menus and looking for Microsoft Excel.
* After you have Excel open, select “Edit” and “Paste”. The WHONET results are now in Excel. You can now use Excel to edit, correct, format, or graph your results.
* Now return to WHONET by clicking on the WHONET icon on the bottom of your screen.
* Click on “Copy graph”.
* Go back to Excel, find an empty part of the spreadsheet, and again choose “Edit”, “Paste”. The graphs are simple images that cannot be further edited except for the height and width. For editable graphs, you should save the results to a “File” as described in the next exercise.
* When you are finished, then leave Excel by selecting “File”, “Exit”. Excel will ask if you want to save the results. For purposes of this exercise, you can response “No”.
	1. Saving results to a file. WHONET also has an option for saving results directly to a file, for example to Excel. The formatting is better than with a simple “Copy/Paste”. The output also includes an informative header and all “Row” and “Column” graphs that you can edit manually (colors, fonts, labels, data values, etc.).
* Click on “Save table”. Give a name to the file, for example “E. coli RIS results.xls” and choose “Excel” as the output format. Note that the default location for this new file is C:\WHONET\Output. Click “Save”.
* After the file has been created, WHONET will ask if you want to open the file. Respond “Yes”. Then review the table and graph contents in Excel. When you are finished, you can close Excel file “File”, “Exit”.
* Click on “Save graph”. Give a name to the file, for example “E. coli %Resistant.jpg”. The file will be saved by default to the folder C:\WHONET\Output.
* After the file has been created, WHONET will not ask if you want to open the file.
* From Windows, you can open the file location C:\WHONET\Output. You should see the two files that you have created above. When you are finished, you can close this window and return to WHONET.

1. **One-per-patient options**
	1. In the preceding analysis, WHONET included results from all isolates of *S. aureus* and *E. coli* in the analysis (except for laboratory and quality control isolates). For some analyses, such as isolate listings, including results from all isolates is valuable and appropriate.

However, for calculations of %Resistant or %Susceptible, the US Clinical and Laboratory Standards Institute (CLSI) and others recommend taking the “first isolate per patient per species” or the “first isolate with antibiotic results per patient per species” to decrease bias associated with patients who have multiple isolates for a species and often higher rates of resistance than for patients with only one isolate.

* From the main analysis screen, click on “One per patient”. You will see that WHONET offers several strategies for handling multiple patient isolates, including “First isolate only”, ”First isolate with antibiotic results”, “Most resistant result for each antibiotic”, and others.
* For this exercise, select “First isolate with antibiotic results”. Click on “OK” to return to the main analysis screen.
* Click on “Begin analysis”. You will notice now that WHONET is displaying results of 76 patients with *Staphylococcus aureus* tested for cefoxitin (in comparison to 86 isolates in an earlier exercise), and 10.5% of the first isolates were resistant to cefoxitin (slightly lower than the 11.6% found in the earlier exercise.
* When finished reviewing the results, click “Continue” again to return to the main analysis screen.
1. **Isolate selection criteria**

In the above analyses we analyzed all clinical isolates of *Staphylococcus aureus* and *Escherichia coli*. (By default, WHONET automatically excludes quality control and other laboratory isolates). In many cases, users would like to select a subset of isolates or patients that meet certain criteria, for example, blood isolates from the neonatal intensive care unit or imipenem-resistant Enterobacterales.

* 1. Defining an isolate filter.
* Click on “Isolates” and you will see that you can define criteria for any of the patient, location, sample, or microbiology fields available.
* Double-click on “Specimen type” (or single-click on “Specimen type” and then click on “Define criteria”). You will then see a list of all specimen types on the left.
* Double-click on “Blood” to select this option and click “OK”. “Blood” will now appear in the list on the right. Click on “OK” to return to the previous screen.
* There are a few other options on this screen that you should leave unchanged for this exercise, but they could be useful to you in the future.
	+ Exclude laboratory samples: By default, WHONET does not include the isolates in which the specimen type is equal to “qc” (quality control), “la” (laboratory sample), or “ex” (external quality control) or if the department is equal to “lab”. If you would like to include these results in your analyses, remove the check in this box.
	+ Include isolates that satisfy all of the selection criteria. Include isolates that satisfy at least one of the selection criteria: If you choose the first of these options, WHONET will search for isolates that meet all of the selection criteria, for example “Blood and Imipenem-Resistant”. Or you can select the option “Include isolates that satisfy at least one of the selection criteria, such as “Resistant to Imipenem Or resistant to Meropenem”.
* After you have defined the above selection criteria, click on “OK” to return to the main analysis screen.
* Click on “Begin analysis”, and you will see the results for *Staphylococcus aureus* in blood.
* Click on “Continue”, and you will see the results for *Escherichia coli* in blood.
* Click on “Continue” again, and you will be back at the main analysis screen.
	1. Other options of isolate filters.
* Click on “Isolates” again to review some of the additional features available on this screen. There are no exercises in this section, but the options may be useful for you in your own work.
* At the bottom of the screen, you will see the below options:
* “Exclude laboratory isolates”. By default, WHONET will not include isolates in the analysis where the “Specimen type” is “Quality control (qc)”, “Laboratory (la)”, or “External quality control (ex)” or if the “Department” is “Laboratory (lab)”. You can remove the checkbox, then WHONET will include these isolates in the analysis.
* “Exclude screening isolates”. By default, WHONET will not include isolates in the analysis where the “Specimen type” is “Screening (sc)”, “Screening for MRSA (mr)”, or “Screening for VRE (vr)”. If you remove the checkbox, then these isolates will be included in the analysis.
* Options for multiple criteria. If you choose multiple criteria, WHONET offers two useful options”
* “Include isolates that satisfy all of the selection criteria”, for example “Sex = Female” AND “Specimen type = “Urine” AND “Location type = Outpatient”.
* “Include isolates that satisfy at least one of the selection criteria”, for example, “Imipenem-Disk = Resistant” OR “Meropenem-Disk = Resistant”.
* When you finish reviewing the above, you can click on “OK” to return to the main analysis screen.
	1. Removing selection criteria

After you have created selection criteria, you can also remove the filters.

* Click on “Isolates” to enter this section again. You should see that the filter for “Specimen type = bl” is still defined on the screen.
* You have the choice at the bottom of selecting “Clear this criterion” or “Clear all criteria”.
* For this exercise, click on “Clear all criteria” to remove the selection criteria defined.
* Click on “OK” to return to the main analysis screen.
1. **Analysis options**
	1. WHONET offers a few additional analysis options available not covered earlier. There are no exercises for this section, but the features might be useful for you in your own work.
* From the main analysis screen, click on “Options”.
	+ “Combine disk, MIC, and Etest results”. By default, WHONET will present separate statistics by method, such as “%Resistance for Gentamicin-Disk” results and “%Resistance for Gentamicin-MIC” results. However, for reporting results to clinicians and policymakers, it is useful to combine these results to obtain an overall “%Resistance for Gentamicin”, irrespective of test method. This feature is valuable when a single laboratory performs antibiotic testing with multiple methods. It is also valuable for national network coordinators when some laboratories rely on disk diffusion testing, while others rely on MIC testing.
	+ “Encrypt patient information”. In isolate listings, WHONET will generally display confidential information such as patient name and date of birth. However, it is generally not recommended to share these confidential details outside of the organization. By utilizing this “encryption” feature, WHONET offers the ability to delete or encrypt confidential information to protect confidential details or to shift dates so that true dates are not known. WHONET offers standard options for encryption, but the user has the ability to customize and optimize these for their own local or national purposes.
* When finished reviewing the results, click on “OK” to return to the main analysis screen.
1. **Existing data analysis**
* When you have finished the above exercises, you can click on “Exit” to leave the data analysis screen.
* You can then click on “File” and “Exit” to close the WHONET software.