

Application of advanced analysis in WHONET software and antibiogram typing for phenotypic detection of Staphylococcus aureus

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Abstract

Introduction:-

Staphylococcus aureus is an important bacterial pathogen responsible for a wide range of infections. Methicillin resistant Staphylococcus aureus is defined as a strain of S. aureus that is resistant to all β -lactams including penicillins and cephalosporins⁽²⁾. Methicillin resistant Staphylococcus aureus (MRSA) is known to cause community acquired and nosocomial infection. . This study aims to utilize antibiogram typing to detect various phenotypes of Staphylococcus aureus in a tertiary care setup using WHONET software.

Material and method:-

The study was conducted in Microbiology laboratory of a tertiary care centre from September 2016 – August 2017, after obtaining institutional ethical approval. All samples received for culture and susceptibility testing in laboratory were included. The scatter plot analysis function of WHONET software was used to perform the following:-

- 1) Two antibiotics from same class, Penicillin and Cefoxitin, (B lactam agents) were plotted against each other on a graph, with Penicillin on X axis and Cefoxitin on Y axis.
- 2) Two antimicrobials belonging to two different classes were plotted against each other on a graph, with Cefoxitin (B- lactam agent) on X axis and Ciprofloxacin (fluoroquinolone) on Y axis.

Result:-

Susceptible results were generated in a graphical and tabulated format. The patterns were used to detect 2.1 % Wild phenotypic Staphylococcus, 43.1 % Methicillin Sensitive Staphylococcus (MSSA) and 54.7 % Methicillin resistant Staphylococcus (MRSA) . On comparing antimicrobial drugs from different categories, 39.3 % were found resistant to Cefoxitin as well as Ciprofloxacin indicating MRSA phenotype due to probable cross resistance.

Conclusion:-

WHONET software is used to generate antibiograms for studying prevalence of various organisms and their drug resistance patterns. It can also be used to perform antibiogram typing using a simple function like scatter plot. Various phenotypes of an organism can be detected in a limited resource set up.

Introduction:-

Staphylococcus aureus is an important bacterial pathogen responsible for a wide range of infections. It is a leading cause of bacteremia and infective endocarditis as well as superficial and deep bone, skin and soft tissue, pulmonary, and device-related infections⁽¹⁾. Methicillin resistant Staphylococcus aureus is defined as a strain of S. aureus that is resistant to all β -lactams including penicillins and cephalosporins⁽²⁾. Methicillin resistant Staphylococcus aureus (MRSA) is known to cause community acquired and nosocomial infection. It poses a major clinical threat, with increasingly high morbidity and mortality⁽³⁾. The gene responsible for causing methicillin resistance in Staphylococcus is mec-A. Laboratory detection of Methicillin resistant Staphylococcus aureus by conventional method takes upto 48 – 72 hours. Polymerase chain reaction takes 5 hours in detecting mec-A gene and is therefore the most recommended method for detecting MRSA^(2,4). However, it may not always be cost effective and availability of detection kits and molecular laboratory in a resource limited facility remains a challenge. Hence phenotypic methods remain the main mode of detecting Methicillin resistance in Staphylococcal infections. This study aims to utilize antibiogram typing to detect various phenotypes of Staphylococcus aureus in a tertiary care setup using WHONET software.

Material and method:-

The study was conducted in Microbiology laboratory of a tertiary care centre from September 2016 – August 2017, after obtaining institutional ethical approval. All samples received for culture and susceptibility testing in laboratory were included. Kirby Bauer disc diffusion method was performed for positive samples and data was entered in WHONET software 5.6 version. Analysis for Staphylococcus aureus from different clinical specimen was carried out. The scatter plot analysis function of WHONET software was used to perform the following:-

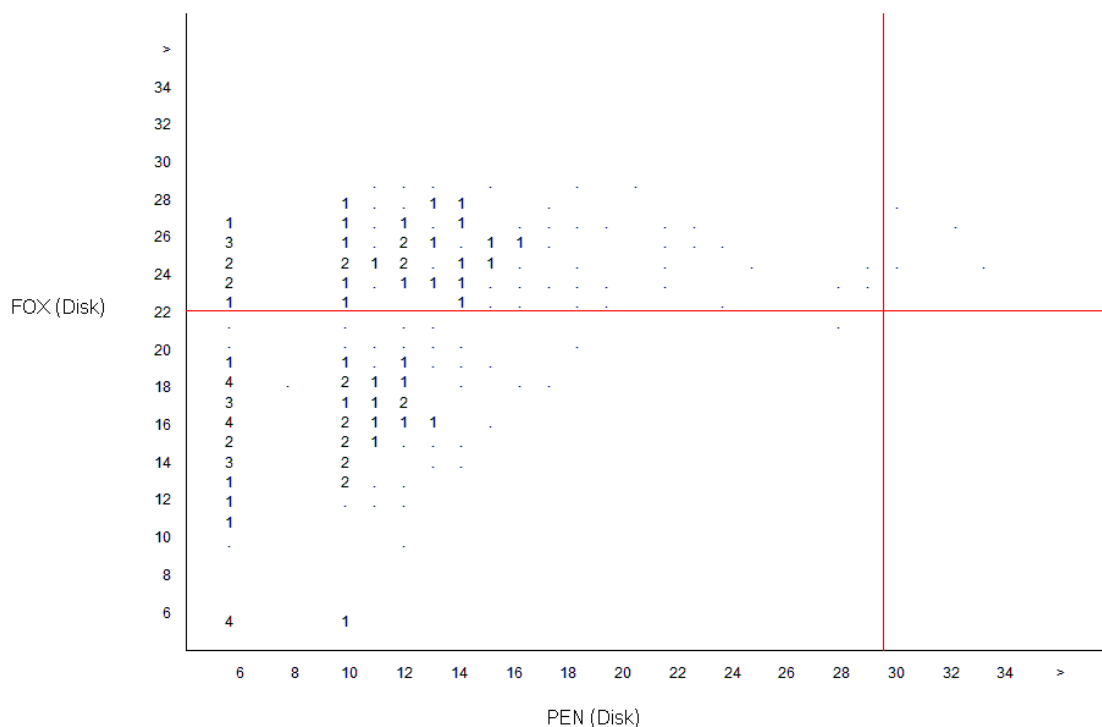
- 3) Penicillin and Cefoxitin, (B lactam agents) were plotted against each other on a graph, with Penicillin on X axis and Cefoxitin on Y axis. Susceptibility results of both antimicrobials were compared using graph number 1 and table number 1.
- 4) Two antimicrobials belonging to two different classes were plotted against each other on a graph, with Cefoxitin (B- lactam agent) on X axis and Ciprofloxacin (fluoroquinolone) on Y axis. Susceptibility results of both antimicrobials were compared using graph number 2 and table number 2.

Result:-

Graph number 1, shows the zone diameter distribution of isolates tested against both penicillin and Cefoxitin. Numbers in the graph represent percentage of isolates, and the red lines indicate the interpretative breakpoints.

- **Upper right-hand corner:-** There are a number of isolates with large zone diameters for Penicillin ($\geq 30\text{mm}$) and large zone diameters for Cefoxitin ($\geq 22\text{mm}$). These isolates are susceptible to both agents – this area represents the traditional wild-type phenotype for *S. aureus*.
- **Lower left-hand quadrant:-** This part of the graph represents isolates resistant to both drugs; these would be the MRSA (methicillin resistant *Staphylococcus aureus*) isolates.
- **Upper left-hand quadrant:-** The greatest number of isolates are in this quadrant. Such isolates are resistant to penicillin (to the left of the red line), but susceptible to cefoxitin (above the red line). This is the classical phenotype for beta-lactamase (penicillinase) producing *S. aureus*.
- **Lower right-hand quadrant:-** Fortunately, there are none to rare isolates in this area, representing penicillin-susceptible and cefoxitin-resistance. These are likely attributable to an error in the laboratory (poor quality reagents, test performance, measurement, data entry)

Graph number 1:- Comparing efficacy of two antibiotics from the same class



X axis:- Penicillin disc

Y axis:- Cefoxitin disc

Results shown in graph number 1, are presented in table number 1 for better understanding, using a quantitative scatterplot, but using the R, I, S (Resistant, Intermediate, Sensitive) categories. Three major subtypes of *S. aureus* are shown in table 1 as follows:-

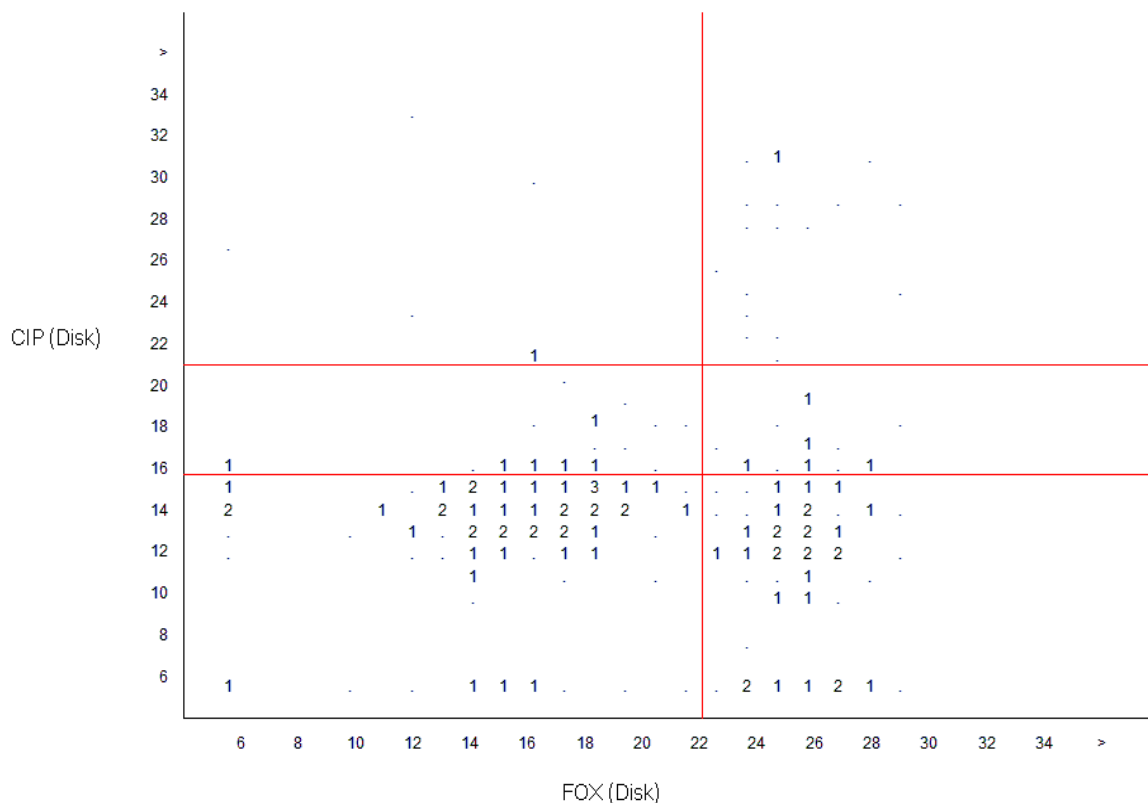
Table 1:- Comparing efficacy of two antibiotics and detecting rates of various phenotypes of Staphylococcus aureus

		PEN (Disk)		
		R	I	S
FOX (Disk)	S	43.1%		2.1%
	I	0.1%		
	R	54.7%		

- 2.1% of the isolates have the wild-type phenotype, sensitive to Penicillin and Cefoxitin (PEN-S, FOX-S)
- 43.1% have the penicillinase-producer phenotype. Resistant to Penicillin and sensitive to Cefoxitin. (PEN-R, FOX-S, MSSA)
- 54.7% have the MRSA (methicillin resistant Staphylococcus aureus) phenotype, Penicillin resistant and Cefoxitin resistant. (PEN-R, FOX-R).
- 0.1% represents intermediate results to Cefoxitin and resistant to Penicillin, likely attributable to a laboratory error as CLSI guidelines do not have intermediate category for Cefoxitin.

Scatter plot analysis of WHONET software was also used to compare two drugs belonging to different classes. Most methicillin resistant Staphylococcus aureus isolates (cefepime-resistant) were also resistant to ciprofloxacin, a commonly observed epidemiological finding in many institutions. Graph number 2 shows the results of the same in this study.

Graph number 2 :- Comparing efficacy of two antibiotics belonging to different classes



X axis:-Cefoxitin disc

Y axis:- Ciprofloxacin

- **Upper right quadrant:-** This area represents strains of Staphylococcus which are sensitive to Cefoxitin as well as Ciprofloxacin, Methicillin sensitive Staphylococcus aureus (MSSA).
- **Middle quadrant:-** This area represents least amount of strains which may fall in the intermediate category to Cefoxitin as well as Ciprofloxacin.
- **Lower Left quadrant:-** This area represents the phenotype of Staphylococcus aureus which is resistant to Cefoxitin as well as Ciprofloxacin. (MRSA phenotype due to probable cross resistance)

Results shown in graph number 2, are presented in table number 2 for better understanding, using a quantitative scatterplot, but using the R, I, S (Resistant, Intermediate, Sensitive) categories.

Table 2 :- Comparing efficacy of two antibiotics and studying epidemiology of cross resistance between them

	S	1.9%	6.9%
CIP (Disk)	I	13%	0.2%
	R	39.3%	32.8%
		R	I
			S
		FOX (Disk)	

- As shown in table 2, 6.9 % represents strains of Staphylococcus which are sensitive to Cefoxitin as well as Ciprofloxacin (MSSA)
- 0.2 % fall in the intermediate category to Cefoxitin as well as Ciprofloxacin. 13 % is intermediate to Ciprofloxacin but resistant to Cefoxitin.
- 5.9 % is sensitive to Cefoxitin but intermediate to Ciprofloxacin.
- 39.3 % represents the phenotype of Staphylococcus aureus which is resistant to Cefoxitin as well as Ciprofloxacin. (MRSA phenotype which represents probable cross resistance)

Discussion:-

Methicillin-resistant Staphylococcus aureus (MRSA) is emerging globally and becoming a leading cause of bacterial infections in both health-care and community. There is remarkable

geographical variation in the load MRSA infection due to different antibiotic policies and infection control practices.

Methicillin-resistant *S. aureus* isolates are often resistant to other classes of antibiotics (through different mechanisms) making treatment challenging which has led to the search for newer antimicrobials⁽⁶⁾. Antibigram typing is a method that helps in detection of various phenotypes of an organism based on their antibiotic susceptibility pattern. Antibiotic susceptibility testing is routinely done in any laboratory and using this method for studying prevalent phenotypes in any institute would give the first sign of an outbreak or epidemic in a hospital. As shown in table 1, the two antibiotics compared (penicillin and cefoxitin) are from the same class of drugs (beta-lactams). Such comparison helps in studying mechanisms of resistance and quality assurance. As shown in table 2, maximum phenotypes of *Staphylococcus* that are resistant to Cefoxitin also showed resistance to Ciprofloxacin. These phenotypes represents cross resistance to both antibiotics. Using scatter plot function for generating analysis of different antibiotics in this way is easily available from the WHONET tutorials.

Conclusion:-

WHONET software is majorly used by Microbiology laboratories for creating and distribution of antibiograms among clinicians. However, it can also be used to do antibiogram typing using a simple function like scatter plot. Performing such analysis will not only help in studying various phenotypes but will also help in formation of antibiotic policy. This will further guide the clinician for empirical use of first and second line drugs, while the culture and susceptibility report is awaited.

References:-

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