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Role of Whonet Software Beyond Antimicrobial Resistance in Clinical Microbiology

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ABSTRACT

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Whonet software introduced by WHO for surveillance of antimicrobial resistance. The scope of the study is that incorporation of quality control alerts in the software which leads to minimal laboratory errors, provides the clinicians therapeutic comments about antimicrobial therapy. Real time data entry of patient, sample details, microscopy and the culture workup were also entered. The antimicrobial susceptibility test results were fed directly in the Whonet software with a digital calliper. A scatter plot, a two-dimensional graph in which the points corresponding to two related or unrelated drugs were graphed and observed for positive and negative correlation of resistance pattern. Each antimicrobial was compared with all the other antimicrobials for cross resistance pattern and a consolidated checker-board table was created for all scatterplots. Whonet is used for the comparison of NS1 antigen with IgM antibody test results and thereby enabling the laboratory to get the feedback for the dengue testing policy regarding the need for NS1 antigen or IgM antibody or both. This software is more than a laboratory information system, that helps the laboratory technologist for proper documentation and reporting.

Introduction

Whonet is a computerized microbiology laboratory data management and analysis program introduced by World Health Organization for the surveillance of antimicrobial resistance worldwide. The software can help the microbiology laboratory in collating and analysis of antimicrobial susceptibility pattern results and guiding the clinicians for empirical therapy, monitoring the trend of antimicrobial resistance, helps in antimicrobial policy decisions

and preventive measures. Whonet can be used in a resource limited laboratory setting, facilitates by sharing and analysis of data among various levels of laboratories as part of national antimicrobial resistance surveillance program. The cumulative antibiograms obtained from various hospitals may help in formulating antimicrobial policies and control measures at national level. Besides, the Whonet software can facilitate the laboratory in the pre-analytical, analytical, and post analytical activities such as

Standardized data entry with user defined data fields and customized code listing.

Integrating a standard laboratory work up like culture and identification with the patient details, accurate entry of antimicrobial Susceptibility Test (AST) results with USB SPB output and incorporation of the quality control activities such as AST results with the ATCC strains.

Customized clinical reporting with all demographic particulars, microscopic findings, culture interpretation, antimicrobial susceptibility test results. The incorporation of alerts in clinical reports that help the clinicians for antimicrobial stewardship, hospital infection outbreak detection and quality control alerts helping the laboratory for identifying the errors.

This study was carried out with the primary intention of using Whonet as a laboratory software for all diagnostic activities in bacteriology and serology in addition to antimicrobial resistance survival.

The scatterplot tool to analyse the cross resistance pattern can be used to obtain cross resistance patterns between all antimicrobials and a consolidated checkerboard type of table can be created that can be used as a ready reckoner for analysing the cross resistance pattern and may help in guiding the antibiotic policy and stewardship.

The main objectives of this study to utilization and application of whonet software in clinical microbiology in a broader perspective in addition to antimicrobial susceptibility.

Materials and Methods

Laboratory Configuration and Data entry

Based on the model laboratory configuration in the Whonet the following additional data field were created: a) clinical details-related to fever, general symptoms and signs and specific system wise symptoms and signs (Central Nervous System,

urogenital etc.). It was possible to enter the data in real time every day like a laboratory information system directly in the software: name of the patient, hospital and sample identification number, clinical history, and specimen type. The laboratory workup details such as direct gram stain examination, the culture workup like, colony morphology and colony microscopy and the biochemical results were entered in real time. The antimicrobial susceptibility test results were entered directly in the Whonet software with USB SPB output. Regarding serology laboratories, additional data fields such as HIV antibody, HBSAg, HCV antibody, Treponemal & Non treponemal antibody fields Finally, consolidated lab registers were created in the Whonet software itself including all specimen types and all configured laboratories separately for culture and serology and updated every day.

Analysis

The software primarily meant for analysis of antimicrobial susceptibility results like organism wise susceptibility pattern was used for the analysis of additional parameters such as correlation of the culture results with the direct gram smear microscopy, comparison of antimicrobial drug resistance profile among pathogens and commensals, Cross resistance pattern among selected antimicrobials using scatterplot. Scatter plot, a two-dimensional graph in which the points corresponding to two related or unrelated drugs were graphed and observed for positive and negative correlation of resistance pattern. Each antimicrobial was compared with all the other antimicrobials for cross resistance pattern and a consolidated checkerboard table was created for all scatterplots.

Results and Discussion

The list of various culture and serology laboratories configured in WHONET (Fig.1) shows that the software is versatile with the ability to configure many laboratories with different functions. In addition to the data fields already available in the Whonet software, newly configured data fields such as clinical details, e.g., fever clinical and culture

interpretation with the corresponding code lists are shown in figure: 2a, 2b & 2c respectively

The laboratory workup details-Direct microscopy - e.g., Gram smear with data fields (pus cells & organisms) with code listing, (Fig: 3a & 3b) show that the software is able to integrate microscopy with culture enabling accurate interpretation of the isolated organism as pathogen or commensal.

The culture workup details with colony morphology on media e.g., blood agar and biochemical reaction e.g., Triple Sugar Iron Agar with corresponding code listing (fig.4a & 4b) enables the laboratory technician to adhere to standard operating procedure with documentation.

Besides the culture workup serology laboratories such as dengue NS1 Ag/IgM ELISA, ASO-Rheumatoid Factor-CRP, HIV Antibody -HBsAg-HCV Antibody were also configured. The data fields of Widal and Dengue serology laboratory with the code listing (fig: 5) reveal the flexibility in configuration of laboratories with different functions.

In the quality control laboratory, it was possible to record the antimicrobial susceptibility pattern for the ATCC strains as per the acceptable zone diameter ranges different from the routine antimicrobial susceptibility pattern for the clinical isolates.

Besides the antimicrobials with discordant zone diameter ranges for ATCC strains such as *Escherichia coli* atcc25922 e.g., Ceftazidime (CAZ) zone diameter 22 mm range (22-29) and piperacillin tazobactam with ZD 22 mm range (25-33) could be notified as “S@” and “I@” respectively for

corrective action (Fig : 6)

The percentage susceptibility (antibiogram) essential for the antibiotic policy and stewardship can be easily obtained from the Whonet software (figure7). The scatterplot tool to analyse the cross resistance pattern can be used to obtain cross resistance patterns between all antimicrobials and a consolidated checkerboard type of table can be created that can be used as a ready reckoner for analysing the cross resistance pattern and may help in guiding the antibiotic policy and stewardship.(Figure 8a,8b)

Another valuable Whonet tool is analysis of the resistance profile to detect the multidrug resistance (MDR) or extremely drug resistance (XDR) or pandrug resistance (PDR).(figure:9)

Analysis of the serological test with reference to Dengue testing (figure 10) shows the comparison of NS1 antigen with IgM antibody results and thereby enabling the laboratory to get the feedback for the dengue testing policy regarding the need for NS1 antigen or IgM antibody or both.

Thus the WHONET software - a tool primarily intended for antimicrobial susceptibility pattern data entry and analysis of antimicrobial resistance can be extended to a variety of applications as a laboratory software for bacteriology, serology and also molecular biology. The software is easily customisable, enabling the laboratory to create additional data fields such as clinical details- general and specific system wise symptoms and signs (respiratory, central nervous system, urogenital etc.) and integrate direct gram microscopy, culture work up, biochemical results and quality control.

Fig.1

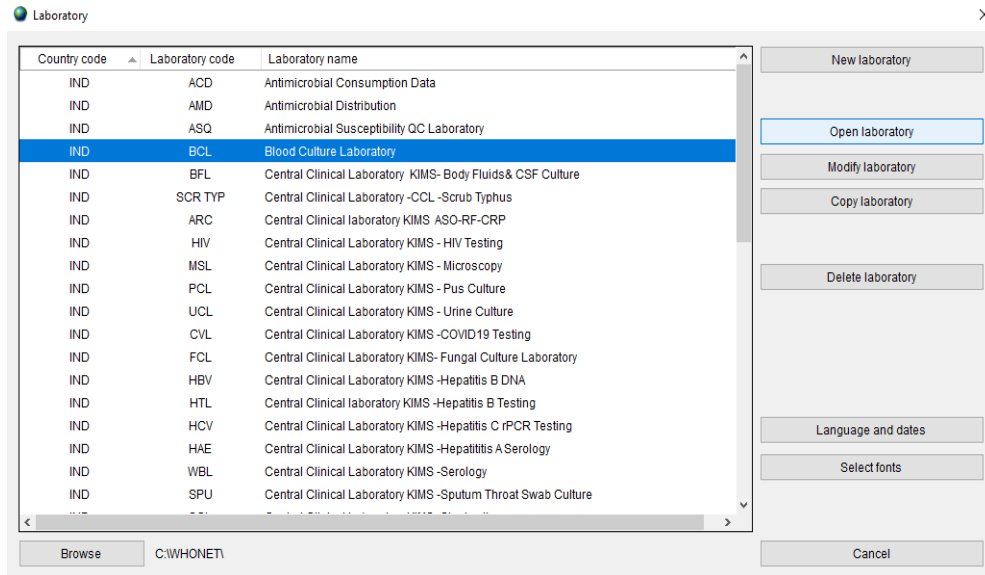


Fig.2a Clinical detail-fever

The screenshot shows a clinical detail form for fever. It includes sections for Patient information, Location, and Specimen. The 'Fever clinical' field is set to 'fev 4-7d'. A legend on the right lists various fever codes and their descriptions.

Patient Information:

- Origin: Human
- Full name: Sharvananth
- Age: 9
- Sex: m
- Identification number: 202109060074
- Central lab.number: 208936
- Microbiology number: 1885
- Patient Category: PAID
- Fever clinical: fev 4-7d
- General clinical: []
- Paediatric clinical: []
- Obs.Gyn clinical: []

Location Information:

- Institution: KIMS
- Date of admission: []
- Location: ped wd
- Location type: in
- Department: Pediatrics
- Reference no: []
- Condition of specimen: Good

Specimen Information:

- Specimen number: 162
- Specimen type: bl

Fever Legend:

Code	Description
fev <=3	Fever <=3days
fev >28	Fever >28 days
fev 14-21	Fever 14 -21days
fev 21-28	Fever 21-28 days
fev 4-7d	Fever 4-7days
fev 7-14	Fever 7-14days
fev uk	Fever -unknown duration
no fev	No fever
no fev	No fever

Fig.2b Culture interpretation-blood with code listing

Specimen		Search	
Specimen number	22	Specimen type	bl
Specimen date	1-Feb-2021	Local specimen code	
Specimen time	5.36pm	Reason	
Received Date	2-Feb-2021	Culture Bottle ID	
Received Time	9.15am		
Microbiology		Code Description	
Test name		Cand Invas	Candidemia -probabe invasive infection
Initial report		con cul	Contaminated culture
Final Report		con spec	Contaminated specimen
Organism	sab Salmonella Paratyphi B	Diphth	Diphtheroids -Normal skin flora ; contaminated specimen
Organism2		E.coli 1	E.coli bacteremia due to probable UTI or wound infection
MRSA		E.coli NB	E.coli bacteremia -Probable neonatal septicaemia
Vancomycin screen plate		enbac	Enterobacter sp. bacteremia due to probable UTI or wound infection
VRE		Encoc2blcul+	Enterococcal bacteremia - probable Subacute bacterial endocarditis
ESBL		Encoc noso	Enterococcal bacteremia- due to ? centraliv cannulation
Blood culture interpretation	Sal.paratyphiB Salmonella paratyphi B Enteric fever	Encoc NB	Enterococci -probable neonatal septicaemia
Interpretation -2		Encoc 1blcul+	Enterococci -probable normal skin flora
Comment		H.infl	Haemophilus influenzae bacteremia probable pneumonitis or meningitis
Comment		Kleb.pneu 1	K.pneumoniae bacteremia due to probable UTI or wound infection
Widal titre:Sty O H		Kle pne NB	K.pneumoniae bacteremia -Probable neonatal septicaemia
		normal flora	Normal skin flora ; contaminated specimen
		pneumo	Pneumococcal bacteremia probable pneumonitis or meningitis
		Pro GBS bacteria	Probable Group B Streptococcal bacteraemia
		Proteus sp 1'	Proteus sp. bacteremia due to probable UTI or wound infection
		Sal.paratyphi A	Salmonella paratyphi A Enteric fever

Fig.2c Culture interpretation- pus with different code listing

Microbiology		Code Description	
Test name		Necf1 Ec Kle A.	? Type 1 Necrotizing fasciitis due to Esch coli/Klebsiella sp and Anaerobic streptococci
Dark ground microscopy		Necf2 Spy	? Type 2 Necrotizing fasciitis due to S.pyogenes
Organism	sau Staphylococcus aureus ss. aureus	Necf1 Sau Spy ...	? Type 1 Necrotizing fasciitis due to S.aureus,S.pyogenes and Bacteroides
Growth		cand AMther	Candida growth-probably due to broad spectrum antimicrobial therapy
Organism2		cul neg path	Culture Negative for Pathogens
Pus culture interpretation	Def path Definite pathogen	Def bac Vag	Definite bacterial vaginosis
Interpretation-2		Def Can Vag	Definite Candidial vaginitis
Interpretation		Def Ecoli pyo	Definite E.coli pyogenic infection
Initial report		Def Kleb pyo	Definite Klebsiella pyogenic infection
Final report		Def path	Definite pathogen
Comment		Def Pseud pyo	Definite Pseudomonas pyogenic infection
Comment		Def S.aureus p...	Definite Staph.aureus pyogenic infection
Beta-lactamase		XDR res colistin	Extremely Drug Resistant organism -may respond to Colistin
ESBL		Nor vag smear	Normal vaginal flora
MRSA	-	Pro ana str	Probable anaerobic streptococcal pyogenic infection
Antibiotic panel	All antibiotics	Pro bac Vag	Probable bacterial vaginosis
		Pro Can vag	Probable Candidial vaginitis
		Pro col wou	Probable colonizer of the wound & not a pathogen
		Prost organ	Probable prost...

Fig.3a Direct Gram smear microscopy data fields with pus cells code listing.

Specimen		Received Time		Caliper		Clear	
Specimen number	137	Local specimen code		Search			
Specimen date	4-Mar-2021	Condition of specimen	Good	[]			
Specimen time	12:55pm	Report date	6-Mar-2021	Code			
Specimen type	ps	Report time	10:10am	Description			
Specimen site		Reason		1+ <5/10 x field (Few)			
Received Date	4-Mar-2021			Pus Pc Few <5/100 x field (Few)			
Microscopy		Gram negative bacilli		3+ >10/10 x field (many)			
Direct microscopy	Gram	Gram positive yeasts	nil	Pus Pc man >10/100 x field (many)			
Squamous epithelial cells	nil	Ziehl Neelsen stain		2+ 5-10/10 x field (moderate)			
RBC		Auramine (Fluorescent) stain		Pus Pc mod 5-10/100 x field (moderate)			
Pus cells	Pus Pc man	Modified Kinyoun Nocardia		PMN Bld sp Many PMN ?due to bloody specimen			
Clue cells	Nil	10% KOH mount		Pus Pc - Nil			
Gram positive cocci	pair&ch+++	Trichomonas vaginalis					
Gram negative cocci	nil						
Gram positive bacilli	Nil						

Fig.3b Direct Gram smear microscopy-Gram Positive Cocci and Bacilli code listing

Specimen site		Report time		Code		Description	
Received Date	4-Mar-2021	Reason	10:10am	AcrOra Acridine Orange			
Microscopy		Gram negative bacilli		FlAu Auramine (Iouochrome) stain			
Direct microscopy	Gram	Gram positive yeasts	nil	FM & ZN Fluorescent & Ziehl Neelsen Microscopy			
Squamous epithelial cells	nil	Ziehl Neelsen stain		Gie Giemsa stain			
RBC		Auramine (Fluorescent) stain		GieBS Giemsa stained blood smear			
Pus cells	Pus Pc man	Modified Kinyoun Nocardia		Gram & ZN Gram & Ziehl Neelsen			
Clue cells	Nil	10% KOH mount		Gram & mod Gram & Modified Ziehl Neelsen			
Gram positive cocci	pair&ch+++	Trichomonas vaginalis		Gram Gram stain			
Gram negative cocci	nil			Iod Iodine wet mount			
Gram positive bacilli	Nil			KOH KOH wet mount			
Microbiology				LPCB LPCB mount			
Test name				sa lwet Saline wet mount			
Dark ground microscopy				TZK Sme Tzank Smear (Giemsa Stain)			
				ZN Ziehl Neelsen			
				ZN mlep Ziehl Neelsen(M.leprae)			
				ZN mod Ziehl Neelsen(modified)			

Specimen site		Report time		Code		Description	
Received Date	4-Mar-2021	Reason	10:10am	chains+ in chains+ (few)			
Microscopy		Gram negative bacilli		chains++ in chains++ (Moderate)			
Direct microscopy	Gram	Gram positive yeasts	nil	chains+++ in chains+++ (many)			
Squamous epithelial cells	nil	Ziehl Neelsen stain		clu+ in clusters + (few)			
RBC		Auramine (Fluorescent) stain		clu++ in clusters ++ (moderate)			
Pus cells	Pus Pc man	Modified Kinyoun Nocardia		clu+++ in clusters +++ (many)			
Clue cells	Nil	10% KOH mount		pair+ in pairs + (few)			
Gram positive cocci	pair&ch+++	Trichomonas vaginalis		pair++ in pairs ++ (moderate)			
Gram negative cocci	nil			pair+++ in pairs +++ (many)			
Gram positive bacilli	Nil			pair&ch+ in pairs & chains + (few)			
Microbiology				pair&ch++ in pairs & chains ++(moderate)			
Test name				pair&ch+++ in pairs & chains +++ (many)			
Dark ground microscopy				pair&clu+ in pairs & clusters + (few)			
				pair&clu++ in pairs & clusters ++(moderate)			
				pair&clu+++ in pairs & clusters +++ (many)			
				nil Nil			

Specimen site		Report time		Code		Description	
Received Date	4-Mar-2021	Reason	10:10am	nocard+ Branching filamentous and beaded Gram positive bacilli+			
Microscopy		Gram negative bacilli		nocard++ Branching filamentous and beaded Gram positive bacilli++			
Direct microscopy	Gram	Gram positive yeasts	nil	nocard+++ Branching filamentous and beaded Gram positive bacilli+++			
Squamous epithelial cells	nil	Ziehl Neelsen stain		diphtheroid few (? diphtheroids)			
RBC		Auramine (Fluorescent) stain		Bacillus + few (? Bacillus sp)			
Pus cells	Pus Pc man	Modified Kinyoun Nocardia		diphtheroi few (? diphtheroids)			
Clue cells	Nil	10% KOH mount		Iacbac + few (? Iactobacilli)			
Gram positive cocci	pair&ch+++	Trichomonas vaginalis		clo perfr in chains - boxcar appearance (Cl.perfringens)			
Gram negative cocci	nil			Bacillus + many (? Bacillus sp)			
Gram positive bacilli	Nil			diphtheroi many (? diphtheroids)			
Microbiology				diphtheroi many (? diphtheroids)			
Test name				Iacbac +++ many (? Iactobacilli)			
Dark ground microscopy				Bacillus + moderate (? Bacillus sp)			
Organism	sau	Staphylococcus aureus ss. aureus		diphtheroi moderate (? diphtheroids)			
				diphtheroi Moderate (? diphtheroids)			
				Iacbac ++ moderate (? Iactobacilli)			
				Nil Nil			
				clos.tetan with terminal spores (Clostridium tetani)			

Fig.5a Widal laboratory- antibody to *S.typhi* O,H, *S.paratyphi* AH,BH with code listing

Location		Department		Code		Description	
Location	fe m1	Department	med	B.abor Pos	Brucellosis due to B.abortus	POSSIBLE	
Location type	in			B.abor Def	Brucellosis due to B.abortus	CONFIRMED	
Specimen		Local specimen code		B.abor Pro	Brucellosis due to B.abortus	PROBABLE	
Specimen number	133	Local specimen code		B.mel Pos	Brucellosis due to B.melitensis	POSSIBLE	
Specimen date	28-Apr-2022	Condition of specimen	Good	b.mel Def	Brucellosis due to B.melitensis	CONFIRMED	
Specimen type	bl	Reason		B.mel Prob	Brucellosis due to B.melitensis	PROBABLE	
Microbiology		WIDAL Tube agglutination		S.ptyA Def	Typhoid fever due to S.paratyphi A	CONFIRMED	
Test name	Widal	WIDAL Tube agglutination		S.ptyA Pro	Typhoid fever due to S.paratyphi A	PROBABLE	
Test 1	Widal			S.ty poss	Typhoid fever due to S.Typhi	POSSIBLE	
Antibody to <i>S.typhi</i> O	> = 400	Titre : > =400		S.ty Prob	Typhoid fever due to S.Typhi	PROBABLE	
Antibody to <i>S.typhi</i> H	> = 400	Titre : > =400		S.ty Def	Typhoid fever due to S.typhi	CONFIRMED	
Antibody to <i>S.typhi</i> AH	25	Titre : 25		Ty Fe neg	Typhoid fever	NEGATIVE	
Antibody to <i>S.typhi</i> BH	50	Titre : 50		S.ptyA pos	Typhoid fever S.paratyphi A	POSSIBLE	
Rise in titre							
Interpretation	S.ty Def	Typhoid fever due to S.typhi	CONFIRMED				
Test 2	S.Typhi Ig	S.typhi IgM IgG Immunochromatography					
IgM antibody to <i>S.typhi</i> <i>S.paratyphi</i>	Pos	Positive					
IgG antibody to <i>S.typhi</i> <i>S.paratyphi</i>	Pos	Positive					
Kit name							

Fig.5b Dengue serology-IgM antibody and NS1 Ag with code listing.

Specimen time		Date sent to ret. lab.		Code		Description	
Specimen time	10.14	Date sent to ret. lab.		Indeter	Indeterminate		
Specimen type	sr	Reason		Neg	Negative		
Received Date	10-Nov-2021	Condition of specimen	Good	Pos	Positive		
Received Time	11.20			Pos ser co	Positive-IgM seroconversion		
Microbiology		Dengue NS1 antigen +Ig M ELISA		Pos low	Positive-low titre		
Report date	10-Nov-2021	Dengue NS1 antigen +Ig M ELISA		Pos mod	Positive-moderate titre		
Report time	17.27			Pos rise	Positive-Rise in titre		
Organism				Pos high	Positive-high titre		
Initial report							
Test method	Den NS1+IgM E	Dengue NS1 antigen +Ig M ELISA					
Dengue -IgM antibody	Pos	Positive					
Kit name	SD Den IgM EL						
Lot number Exp	DEN IgM 5						
Test name	Den IgM	Dengue IgM ELISA					
Dengue NS1 Antigen	Neg	Negative					
Kit name	Den NS1 EL SC	Dengue (NS1 Ag) ELISA Standard Diagnostics					
Lot number Exp	DEN NSAg 3						
Dengue -IgG antibody							
Kit name							

Fig.6 Quality control laboratory-antimicrobial disc with ATCC strains

Microbiology
 Mueller Hinton Agar
 Organism: eco Escherichia coli
 QC range Sensitive @
 QC range Resistant @
 QC range Intermediate @
 Inoculum: 0.5MF
 Incubation temp.: 37°C
 Discs : observation
 Discs : replacement
 MHA Growth QC: Pass
 Cefixime
 Antibiotic panel: Gram negative urine

Print
 Exit
 Calliper Clear

Clinical reports
 <F8> Include or exclude an antibiotic
 <F9> Include all tested antibiotics

Cefotaxime/Clavulanic acid
 CLSI
 30/10ug
 atcc25922
 No QC range for this antibiotic.

Alerts
 WHONET-9 Medium priority
 All organisms
 Penicillins and Beta-lactam+Inhibitor = Discordant results
 Quality control alert - The resistance pattern is rare
 Microbiologist message:
 This is an uncommon resistance pattern. Check for a possible laboratory error.
 WHONET-29 Medium priority
 Enterobacteriaceae

AMK 21 S AMX AMC AMY AMP 21 S
 SAM AZM AZM CFM 23 S CTX 25 I@
 CTC CAZ 22 S@ CCV CRO
 CEP CHL CIP DOX
 GEN 22 S IPM LNZ MEM 28 S
 NAL NET NET NIT 22 S
 OFX TZP 20 I@ TCY 16 S@ TOB 21 S
 VAN

Other
 Comment

Fig.7 Antibiogram of Escherichia coli

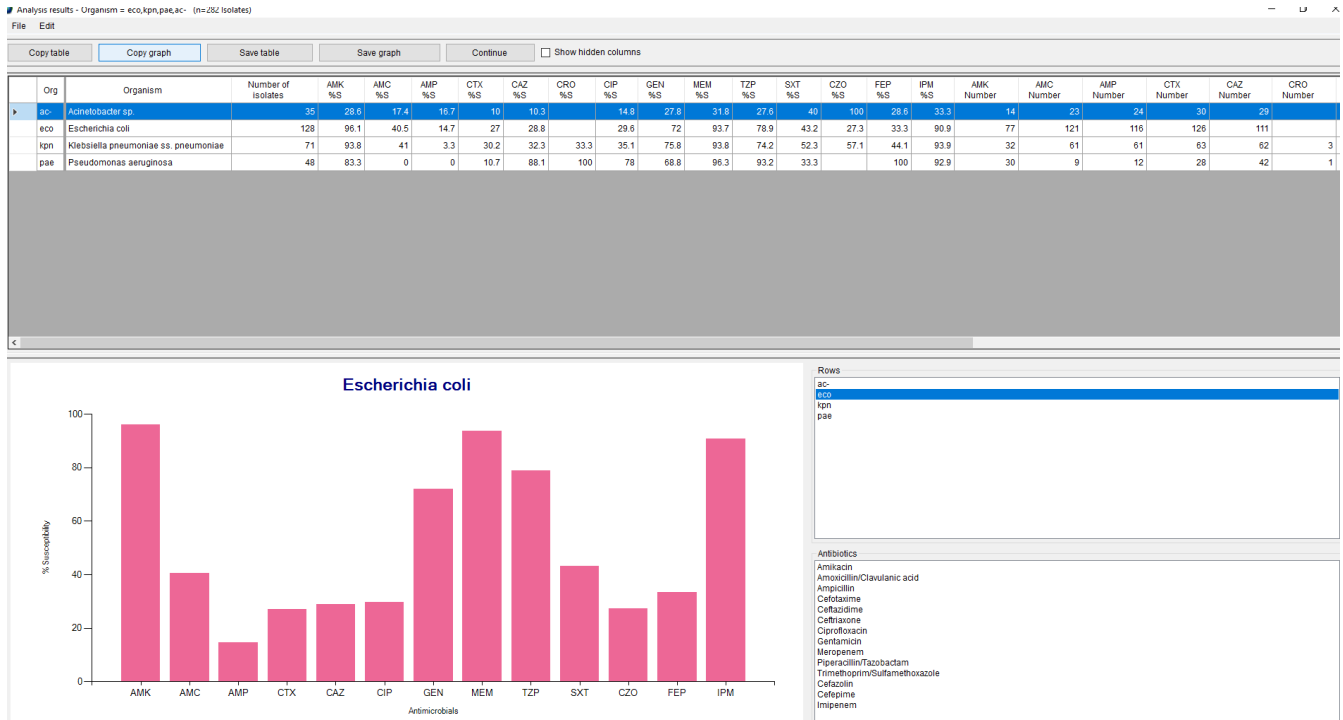


Fig.8a Scatterplot of Antimicrobial cross resistance pattern *Escherichia coli*

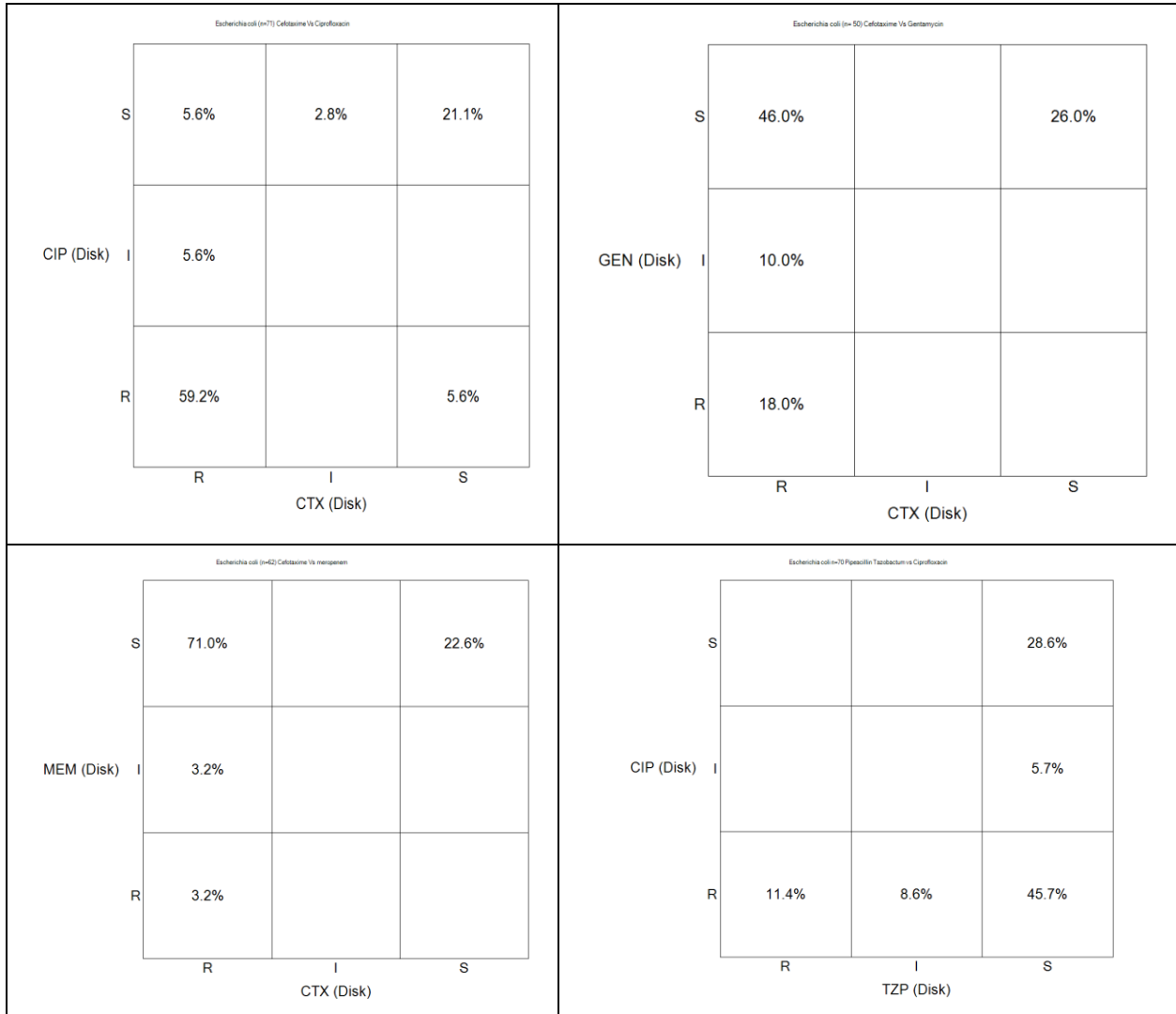


Fig.8b Checkerboard type analysis of scatter plots

Sensitive vs Resistant			Sensitive vs Sensitive						
	AMK	CTX	CAZ	PZT	CIP	SXT	AMC	MER	
AMK		28.9	30.9	82.7	37.5	46.4	45.8	89.2	AMK
		64.5	51.5	5.3	57.5	47.8	43.1	2.7	
CTX	2.6		20.9	24.6	21.1	16.9	20.7	22.6	CTX
	0		0	0	5.6	5.9	5.8	0	
CAZ	4.4	54.5		25.9	16.7	13.6	20	19.2	CAZ
	0	7.3		38.9	6.7	23.3	6.7	0	
PZT	4	12.3	12		28.6	37.7	36.8	77.4	PZT
	0	52.5	0.9		45.7	37.7	1.7	0	
CIP	0	59.2	45	11.4		22.1	18.8	27.8	CIP
	0	5.6	8.3	0		2.9	18.8	0	
SXT	2.9	45.8	33	9.6	45.6		23.7	41	SXT
	89.2	26.3	12.6	3.5	19.1		14.9	0	
AMC	2.8	43	44.8	11.1	40.6	31.6		38.7	AMC
	0	19.8	4.8	33.3	8.7	16.7		0	
MER	0	3.2	3.8	3.2	2.8	3.3	3.2		MER
	5.4	71	55.8	11.3	8.3	47.5	43.5		
	AMK	CTX	CAZ	PZT	CIP	SXT	AMC	MER	
Resistant vs Resistant					Resistant vs Sensitive				

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doi:10.1016/S0377-1237(09)80020-8.
https://wwwnc.cdc.gov/eid/article/1/2/95-0209_article

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