PATHNOSTICS

MOVING CARE FORWARD

The Development of an Integrated Molecular and Phenotypic Method for the Management of Urinary Tract Infections

Dave Baunoch, PHD

Urinary Tract Infections

Cost of Urinary Tract Infections (UTI)

Problem: Urinary Tract Infections cost the US healthcare system

$13B

a year. Without efficacious diagnostic tests, ineffective antibiotics will continue to lead to more resistant microorganisms.

Cost to the Healthcare System

Responsible for

≈10.5

MILLION
office visits/year[1]

UTI complications result in

9-11

DAYS
longer for each hospital stay[2]

Urinary Tract Infections account for

50%

of all
Medicare admissions[5]

Cost to Humanity

Up to **1/3** of infections illustrate resistance to an antibiotic[3]

According to the CDC, **antibiotic resistance** gives rise to at least

2 MILLION INFECTIONS and **23,000** DEATHS/YEAR[4]

1. Flores-Mireles, A. et al. 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*. 13, 5 (2015), 269–284
2. Sheng, W. et al. 2005. Impact of Nosocomial Infections on Medical Costs, Hospital Stay, and Outcome in Hospitalized Patients. J Formos Med Assoc. 104 (2005), 318-326
3. Schmiemann, G. et al. 2012. Resistance profiles of urinary tract infections in general practice--an observational study.BMC urology. 12, 1 (2012), 33.
4. http://www.cdc.gov/drugresistance/threat-report-2013/
5. Strausbaugh, L.J. et al. The Burden of Infection in Long-Term Care 2016 Infect Con and Hosp Epid 2000:21:674-679

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UroSepsis

Problem: Culture has significant limitations including the inability to detect gram-positive organisms.

57.1 %

of patients with gram-positive urosepsis die.

Impact of Urosepsis

50%

Of the sepsis cases in Nursing Homes originate from urinary tract infections

60%

Of patients with sepsis caused by urinary tract infections die[2]

$2.8 B

Total cost of sepsis due to urinary tract infections in the US annually[3]

Impact of an incorrect Diagnosis and Treatment

67.8%

Of patients prescribed the wrong antibiotic on initial treatment died (as compared to 28.7% of patients receiving the right antibiotic)[4]

Initial treatment delay **increases the odds** of admission to ICU up to

1.98

And decreases the overall survival rate[5]

2. Am J Surg. 1999 Apr;177(4):287-90
3. Open Forum Infect Dis. 2017 Winter; 4(1): ofw281
4. J. Antimicrobial chemotherapy. 2008 61, 436-441
5. Supportive Care in Cancer. 2019. 27:4171-4178

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The Problem with Routine Culture and Sensitivity

Problem Statement #1

URINE IS NOT STERILE AND CONTAINS A MICROBIOME

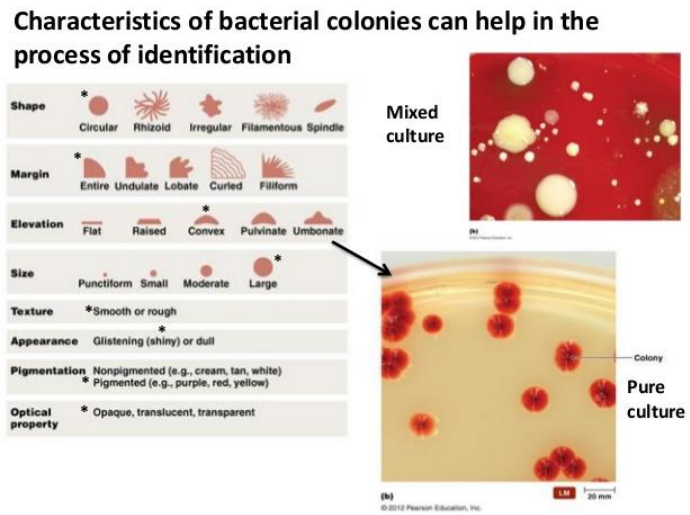
- Urine has historically been viewed to be sterile with the presence of bacteria in the urine seen as an indicator of infection.
- Recent work has clearly demonstrated that the urinary tract contains a microbiome that is characterized by a preponderance of *Lactobacillales* in women and *Corynebacterium*in in men.[1] Often times, the bladder contains additional uropathogenic organisms (including E.coli) that can coexist in a persistent state of asymptomatic bacteriuria.[2]

1. Kim J. Et al. Lactobacillus and Urine Microbiome in Association with Urinary Tract Infections and Bacterial Vaginosis. Urogenit Tract Infect 2018;13(1):7-13 Nicolle, l.
2. The Paradigm Shift to Non-Treatment of Asymptomatic Bacteriuria. Pathogens. 2016 Jun; 5(2): 38.

Traditional Method

Step 1: Identify Organism: 24-48 hours

Step 2: Susceptibility: 24-48 hours



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Problem Statement #2

URINE CULTURE MISSES THE VAST MAJORITY OF POTENTIAL UROPATHOGENS

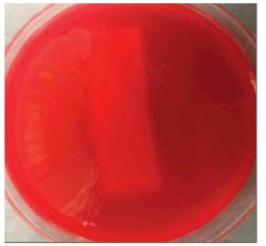
- **Current Methodology** - Developed in the 1950's, the standard method involves applying 1ul of urine onto Blood and MacConkey Agar plates and incubating them at 35 degrees centigrade for 24 hours in the presence of oxygen.[1]

- **What is the Fundamental Problem with culture as a detection method?** From the composition of the agar, to the pH, gas ratio's, and time of incubation culture is a methodology that has been biased for the detection of a subset of pathogens – primarily *E. Coli.*[2]
  - The biased results developed using this methodology often creates findings that are not consistent with the clinical symptoms
  - It is unable to detect slow growing organisms including fastidious and anaerobic organisms as well as most gram-positive organisms
  - Time consuming process that can take up to 72 hours to complete
  - Inherent methodology issues limit the number of organisms reported to no more then 2 with 3 or more considered indications of contamination

Uropathogens detected
Standard urine culture (SUC) versus enhanced quantitative urine culture (EQUC)

Organism	SUC	EQUC
Actinobaculum schaalii	0	5
Aerococcus urinae	1	11
Alloscardovia omnicolens	0	6
Candida albicans	0	2
Candida parapsilosis	0	4
Citrobacter koseri	0	1
Corynebacterium riegelii	0	3
Corynebacterium urealyticum	0	1
Enterobacter aerogenes	1	3
Enterococcus faecalis	1	12
Escherichia coli	***22***	***24***
Klebsiella pneumoniae	3	8
Proteus mirabilis	0	1
Pseudomonas aeruginosa	1	1
Serratia marcescens	0	1
Staphylococcus aureus	1	5
Streptococcus agalactiae	1	10
Streptococcus anginosus	1	25

Adapted from June 9, 2016 ASM Microbe presentation by Alan J. Wolfe, PhD: "Urine Is Not Sterile: Why We Should Care."



Blood agar, 1 µL, 24 hours, aerobic

Blood agar, 100 µL, 48 hours, 5% CO_2

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Problem Statement #3

THE ORGANISMS MISSED BY URINE CULTURE ARE CLINICALLY SIGNIFICANT

The Loyola Study- Pivotal study demonstrating limitations of standard culture in identifying the pathogens associated with UTI's.

- Followed 150 patients who were split into two groups based on whether they believed they were symptomatic for UTI.[1]
- Compared the results obtained when they used standard culture to an enhanced version which had modified growth conditions including an increased incubation time.
- In the group who believed they were symptomatic standard culture detected only 57% of the uropathogens where the enhanced methodology detected 91%.

1. Price, T.K., Dune, T., Hilt, E.E., Thomas-White, K.J., Kliethermes, S., Brincat, C., Brubaker, L., Wolfe, A.J., Mueller, E.R. and Schreckenberger, P.C. 2016. The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. Journal of clinical microbiology. 54, 5 (May 2016), 1216–22

Why is this important?

IN SYMPTOMATIC PATIENTS, STANDARD CULTURE MISSED 2/3 OF ALL POSITIVE PATIENTS

- Outcome
  - Culture positive: 38.5%
    - Failed to see symptoms improve – with standard of care based upon culture results
    - Symptoms worsen
  - Culture negative 67%
    - Failed to see symptoms improve
    - Symptoms worsen

Problem Statement #4

URINE CULTURE MISSES POLYMICROBIAL INFECTIONS

- Wolfe and Brubaker have proposed moving from an E.coli-centric view of urinary tract infections.[1]
  - With an increasing number of studies demonstrating that most urinary tract infections have multiple urinary pathogens present in the same sample, we should begin to shift our thinking away from a monocentric view of urinary tract infections.
- Studies by Price et al, 2016, and Wolfe et al, 2012 have demonstrated that a significant number of cases identified as having only E.coli present actually have multiple species present.[2,3]
  - In the Price et al, 2016 paper 35 of 43 patients had additional species
  - In 25 of 35 cases the additional species were uropathogens
- A simple truth: It is becoming increasingly clear that a significant number of urinary tract infections are polymicrobial in nature. Because of the polymicrobial nature of infections, efficacy of treatment is dropping significantly.

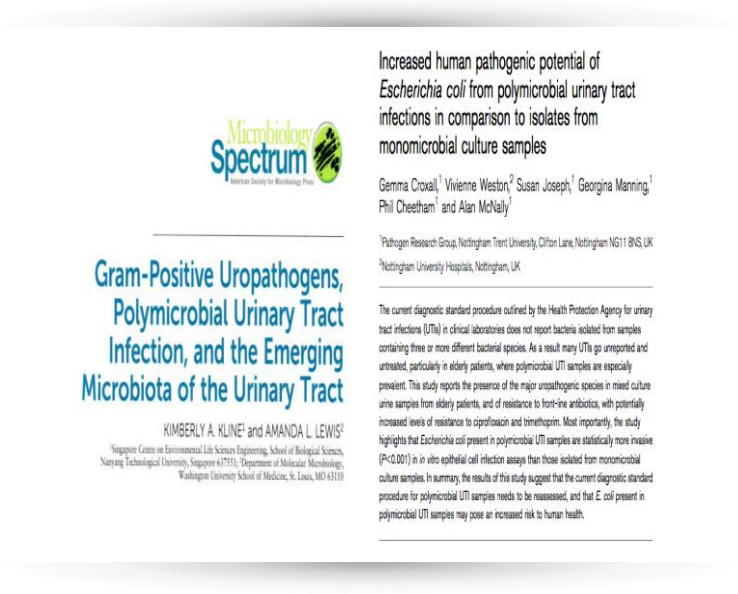
1. Price, T.K., Hilt, E.E., Dune, T.J. et al. Int Urogynecol J (2018) 29: 205. https://doi.org/10.1007/s00192-017-3528-8
2. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE, Brubaker L. 2012. Evidence of uncultivated bacteria in the adult female bladder. J. Clin. Microbiol. 50:1376–1383.
3. Price, T.K., Dune, T., Hilt, E.E., Thomas-White, K.J., Kliethermes, S., Brincat, C., Brubaker, L., Wolfe, A.J., Mueller, E.R. and Schreckenberger, P.C. 2016. The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms. Journal of clinical microbiology. 54, 5 (May 2016), 1216–22

Problem Statement #5

INTERACTION OF BACTERIA IN POLYMICROBIAL INFECTIONS RESULTS IN CHANGES TO ANTIBIOTIC SENSITIVITY

The sharing of metabolic products provides polymicrobial infections an advantage[1]

- In the presence of antibiotics the sharing of metabolic products plays a protective role increasing resistance and virulence
- Testing isolated bacteria may over or under estimate the degree of antibiotic resistance



Kline KA, Lewis AL. Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract. *Microbiol Spectr*. 2016;4(2):10.1128/microbiolspec.UTI-0012-2012. doi:10.1128/microbiolspec.UTI-0012-2012

Problem Statement #6

THE LENGTHY TURNAROUND TIME FOR STANDARD CULTURE AND SENSITIVITY SUPPORTS EMPIRIC TREATMENT AND ANTIBIOTIC RESISTANCE

2018-PUUS Western IRB Number: 20181661 Not yet published.

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Summary of the Problem

ROUTINE CULTURE AND SENSITIVITY

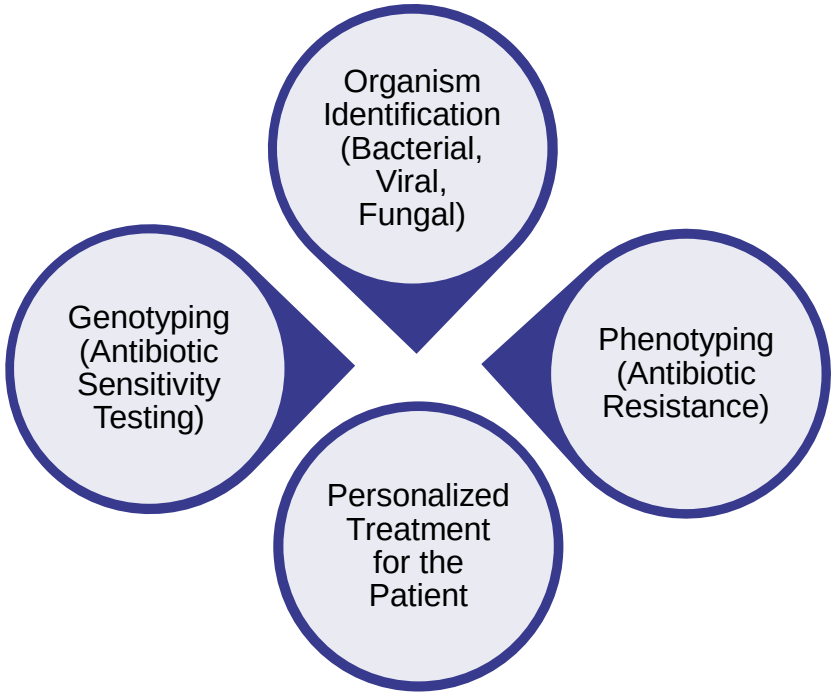
1. Urine is not sterile and contains a Microbiome
2. Urine culture misses that vast majority of potential uropathogens
3. The organisms missed by urine culture are clinically significant
4. Urine culture misses polymicrobial infections
5. Interaction of bacteria in polymicrobial infections results in changes to antibiotic sensitivity
6. The lengthy turnaround time for standard culture and sensitivity supports empiric treatment and antibiotic resistance

The Development of an Integrated Molecular and Phenotypic Method for the Management of Urinary Tract Infections

Guidance UTI

A unique rapid molecular test for both pathogen identification and antibiotic sensitivity providing personalized therapy options that work the first time.

- **Detects 42 Specific Organisms**
- **Pooled Sensitivity for 19 Antibiotics**
- **Higher Sensitivity and Accuracy**



- **Turn Around Time of Less Than 24 Hours**
- **38 Resistance Genes Tested**
- **Detects Polymicrobial Infections**

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KEY:

- Guidance Basic and Guidance Comprehensive
- Guidance Comprehensive only
- Add-on tests

ORGANISMS DETECTED:

BACTERIAL/YEAST ORGANISMS

- *Acinetobacter baumannii*
- *Actinobaculum schaalii*
- *Aerococcus urinae*
- *Alloscardovia omnicolens*
- *Candida albicans*
- *Candida glabrata*
- *Candida parapsilosis*
- *Citrobacter freundii*
- *Citrobacter koseri*
- *Corynebacterium riegelii*
- *Enterobacter aerogenes*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Morganella morganii*
- *Mycoplasma hominis*
- *Mycoplasma genitalium*
- *Mycobacterium tuberculosis*
- *Pantoea agglomerans*
- *Proteus mirabilis*
- *Providencia stuartii*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Ureaplasma urealyticum*

BACTERIAL GROUPS

- Coagulase neg. staphylococci*
- Viridans group streptococci**

SEXUALLY TRANSMITTED ORGANISMS

- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Trichomonas vaginalis*

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Emerging Organisms

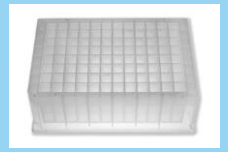
Gram Negative	
Citrobacter freundii	Known to cause several nosocomial infections of the urinary tract (5). Wiping posterior to anterior following a bowel movement and normal intercourse can both bring about C. freundii UTIs.
Citrobacter koseri	Can cause UTI's more frequently in individuals with urinary diversions.
*Enterobacter aerogenes**	E. aerogenes is acquired by unintentional bacteria transmission in hospital settings, and E. aerogenes infections mainly occur when host immunity defenses are already suppressed.
Escherichia coli	Uropathogenic E. coli (UPEC) is one of the leading root causes of UTIs.
Klebsiella oxytoca	Cause UTI's in individuals with catheters.
Klebsiella pneumoniae	UTIs caused by multidrug-resistant K. pneumoniae isolates are a significant public health issue.
Morganella morganii	UTIs are typically connected with complicated features like indwelling catheters.
Pantoea agglomerans	Is associated with catheter-related bacteremia.
Proteus mirabilis	Can cause symptomatic infections of the urinary tract, including cystitis and pyelonephritis, and can appear in cases of asymptomatic bacteriuria.
Providencia stuartii	Are among the most common cause of catheter-associated UTIs, particularly in the elderly with long-term indwelling urinary catheters.
Pseudomonas aeruginosa	Is a common cause of UTIs, and patients with impaired immunity possess a greater risk for colonization by this organism.
Serratia marcescens	Has now been implicated as an etiological agent in a wide range of infections, including UTIs.

Gram Positive	
Actinobaculum schaalii	Is a frequent cause for urinary tract infections amongst the elderly, in children, and in those with underlying urological conditions.
Aerococcus urinae	Can cause simple and complicated UTIs, bacteremia, and endocarditis in elderly adults who have multimorbidity, chronic urinary retention, or indwelling catheters.
Alloscardovia omnicolens	Is hard to identify and one of the organisms identified by Enhanced Quantitative Urine Culture in women complaining of UTI symptoms.
Corynebacterium riegelii	Have been reported to cause opportunistic infections in both immunocompromised as well as immunocompetent patients.
Enterococcus faecalis	Can trigger endocarditis and septicemia, UTI, meningitis, and other infections in people.
Staphylococcus aureus	Has been identified in children with urinary tract abnormalities and vesico-ureteral reflux.
Streptococcus agalactiae	Has been isolated in patients with UTI symptoms.

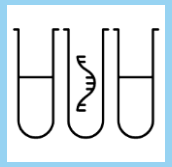
Real-time PCR workflow

DNA Extraction

2-10mL urine sample



Samples undergo extraction



DNA isolated and loaded

Real-time PCR

Samples undergo Real-Time PCR

Results are analyzed by CLS for detection of microbial DNA

ABR

Based on results, samples are set-up for ABR testing.

Antibiotic sensitivity results are obtained through a spectrophotometric assay.

Finalized report is released to client.

Real-time PCR “CHIP”

- Ability to run high numbers of samples simultaneously, with large numbers of targets.
- Isolated DNA and Master Mix is plated onto a nanofluidic plate with 3,072 through-holes.
- Each chip can hold 21-24 samples (18 patient samples and 3 controls) 56 Targets in duplicate
- Estimated Assay Run Time: 2 hours

Software analysis

A. Raw data is gathered through the software for analysis.

- Amplification plots are displayed for individual targets, samples, and subarrays.
- CT's, Amplification scores, and Cq Confidence values are given for each subarray and its 64 wells.

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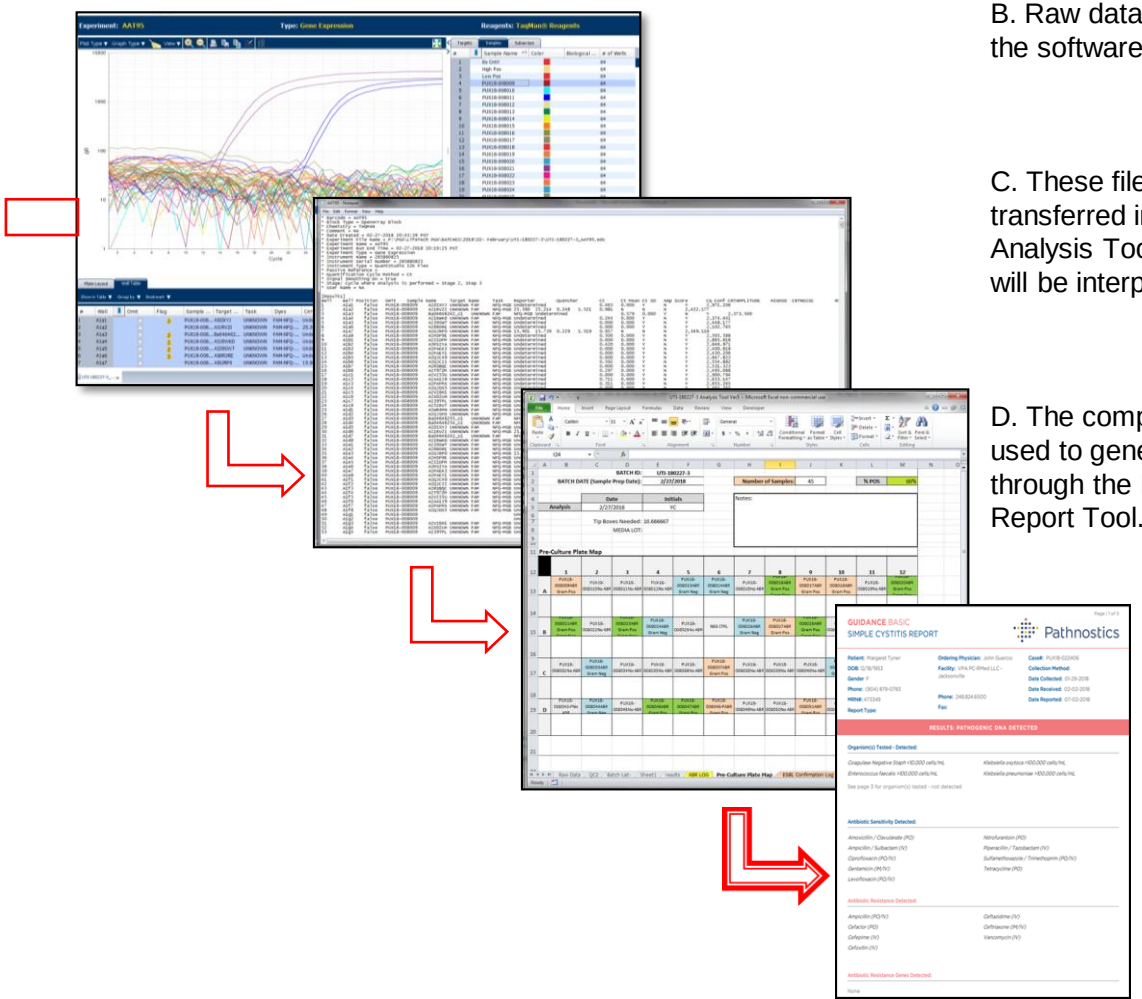

B. Raw data is exported from the software as a (*.txt) file.

C. These files will then be transferred into the Excel Analysis Tool where the data will be interpreted.

D. The completed analysis is used to generate reports through the Excel Integra Report Tool.

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GENOTYPE ANSWERS ONLY PART OF THE COMPLEX PROBLEM OF ANTIBIOTIC RESISTENCE

- Guidance tests for the presence of 38 genes know to be associated with resistance to certain antibiotics
- Does Not Provide the Complete Answer – Why?
  - Limited number of resistance genes that can be identified via molecular assay
  - Gene resistance continuously change
  - Resistance gene may not be active.

Antibiotic Class	Gene	Antibiotic Class	Gene
Macrolide resistance	ErmA + Erm B	Carbapenem resistance	VIM
	ermC		KPC
	mefA		IMP-2 group
Extended-Spectrum-Betalactamase	TEM		IMP-1 group
	CTX-M group 1		OXA-23
	SHV		IMP-16
	VEB		IMP-7
	OXA-1		OXA-72
	CTX-M group 2		OXA-40
	CTX-M group 9		OXA-58
	CTX-M group 8/25		OXA-48
	PER-1		NDM
	PER-2		blaOXA-48
	GES	Tetracycline	TetM
	blaNDM-1		TetB
Quinolone and fluoroquinolone resistance	QnrA	Aminoglycoside	aaC6-aph3
	QnrB		anti-la-aph2
Methicillin resistance	mecA	Trimethoprim/Sulfamethoxazole	drf(A1, A5), sul (1,2)
Vancomycin resistance	vanA1	AmpC resistance	ampC, FOX, ACC
	vanA2		DHA, MOX/CMY, BIL/LAT/CMY
	vanB		

Pathnostics
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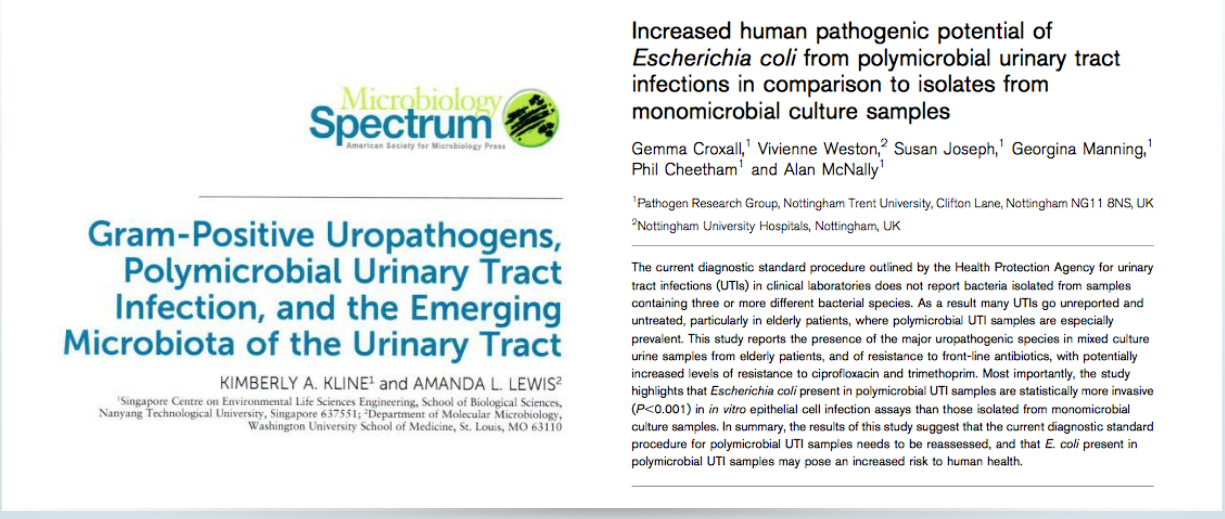
Developing a Methodology for Measuring Pooled Sensitivity

It is becoming increasingly clear that a significant number of urinary tract infections are polymicrobial in nature. Because of the polymicrobial nature of infections, efficacy of treatment is dropping significantly.



Microbiology Spectrum
American Society for Microbiology Press

Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract

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Increased human pathogenic potential of *Escherichia coli* from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples

Gemma Croxall,[1] Vivienne Weston,[2] Susan Joseph,[1] Georgina Manning,[1] Phil Cheetham[1] and Alan McNally[1]

[1]Pathogen Research Group, Nottingham Trent University, Clifton Lane, Nottingham NG11 8NS, UK

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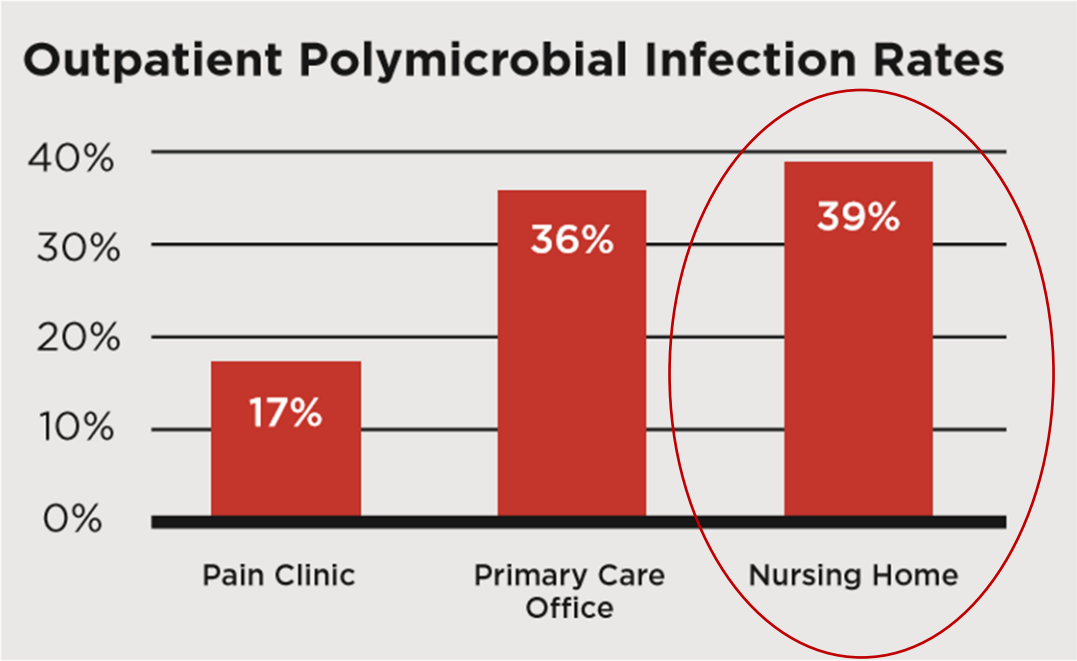
The current diagnostic standard procedure outlined by the Health Protection Agency for urinary tract infections (UTIs) in clinical laboratories does not report bacteria isolated from samples containing three or more different bacterial species. As a result many UTIs go unreported and untreated, particularly in elderly patients, where polymicrobial UTI samples are especially prevalent. This study reports the presence of the major uropathogenic species in mixed culture urine samples from elderly patients, and of resistance to front-line antibiotics, with potentially increased levels of resistance to ciprofloxacin and trimethoprim. Most importantly, the study highlights that *Escherichia coli* present in polymicrobial UTI samples are statistically more invasive ($P<0.001$) in *in vitro* epithelial cell infection assays than those isolated from monomicrobial culture samples. In summary, the results of this study suggest that the current diagnostic standard procedure for polymicrobial UTI samples needs to be reassessed, and that *E. coli* present in polymicrobial UTI samples may pose an increased risk to human health.

Polymicrobial Infections are in the Community



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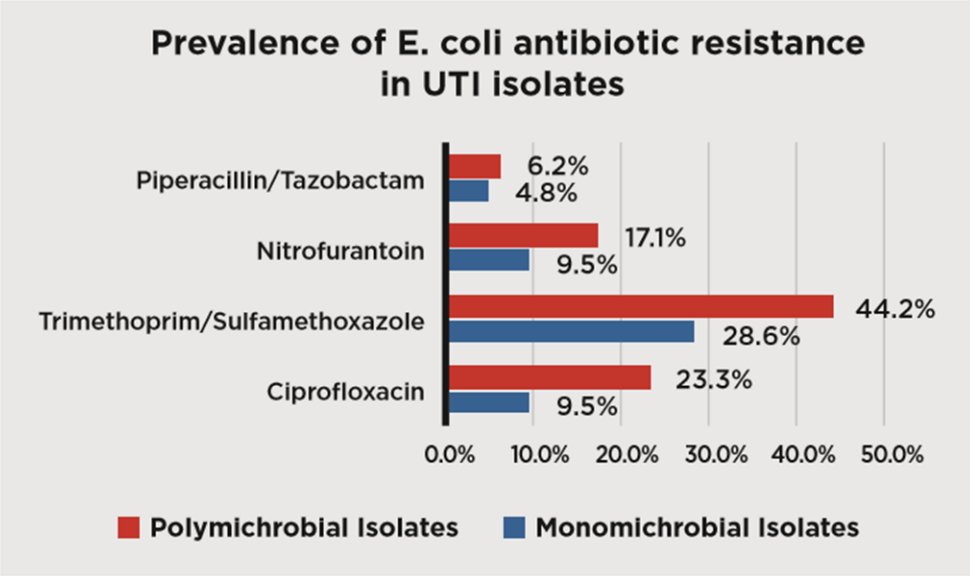
Resistance Higher For Polymicrobial Infections



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Pathnostics

Polymicrobial Interactions Change MIC Levels

Increased human pathogenic potential of *Escherichia coli* from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples

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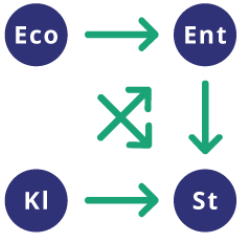
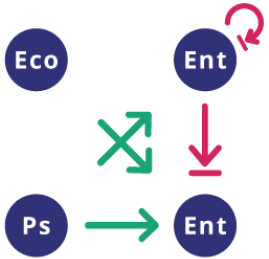
Table 1. Prevalence of antibiotic resistance in UTI isolates

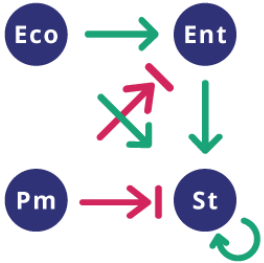
'Poly' refers to strains of polymicrobial infection origin, whilst 'mono' refers to strains of monomicrobial infection origin. –, Particular species/antibiotic combination not tested.

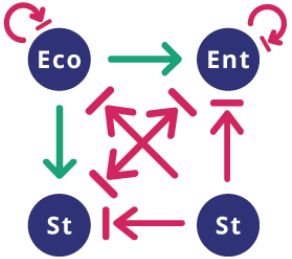
Antibiotic concentration (μg ml^{-1})	*Escherichia coli* (%)		*Enterococcus faecalis* (%)		*Proteus mirabilis* (%)		*Staphylococcus aureus* (%)		*Pseudomonas aeruginosa* (%)	
	Poly ($n=129$)	Mono ($n=21$)	Poly ($n=110$)	Mono ($n=4$)	Poly ($n=56$)	Mono ($n=1$)	Poly ($n=18$)	Mono ($n=4$)	Poly ($n=46$)	Mono ($n=5$)
Gentamicin (2)	12.4	4.76	–	–	5.3	0	4.5	0	–	–
Cefotaxime (1)	17.8	14.29	–	–	33.9	100.0	–	–	–	–
Ceftazidime (1)	18.6	9.52	–	–	35.7	100.0	–	–	–	–
Meropenem (2)	0	0	15.5	0	0	0	–	–	–	–
Piperacillin–tazobactam (16)	6.2	4.76	4.5	25.0	23.2	100.0	–	–	–	–
Co-amoxiclav (32)	5.4	0	2.7	0	16.1	100.0	0	0	–	–
Trimethoprim (2)	44.2	28.57	–	–	89.0	0	22.2	0	–	–
Ciprofloxacin (4)	23.3	9.52	28.2	0	0	0	55.5	50.0	–	–
Cefradine (32)	28.7	19.05	–	–	55.3	100.0	50.0	25.0	–	–
Nitrofurantoin (32)	17.1	9.52	10.9	0			11.0	0	–	–
Amoxicillin (32)	45.0	42.86	7.3	0	37.5	100.0	66.6	75.0	–	–
Gentamicin (10)	–	–	–	–	–	–	–	–	2.17	0
Piperacillin–tazobactam (85)	–	–	–	–	–	–	–	–	0	0
Ceftazidime (30)	–	–	–	–	–	–	–	–	2.17	0
Meropenem (10)	–	–	–	–	–	–	–	–	2.17	0
Ciprofloxacin (1)	–	–	–	–	–	–	–	–	4.35	0

Mutualism: Cross Feeding









4 Examples of Stable Communities

- Positive Interactions (cross feeding)
- Negative Interactions (nutrient competition)
- Eco — E. coli
- Ent — Enterococcus spp.
- Ps — Pseudomonas spp. (P. aeruginosa, P. fluorescens)
- Kl — Klebsiella spp (K. pneumoniae, K. oxytoca)
- Pm — P. mirabilis
- St — Staphylococcus spp (S. aureus, S. haemolyticus, S. capitis)

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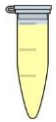
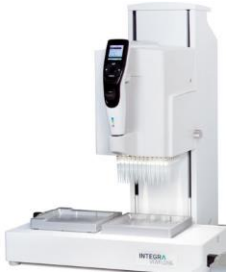
Antibiotic Sensitivity Testing

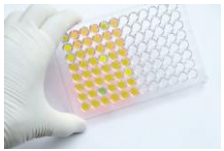
Upon completion of UTI Molecular Testing, results are used to set up Antibiotic Sensitivity Testing for each sample.

Overview:



1mL of urine is processed by Micro Dept. Sample is incubated 6-16 hours.





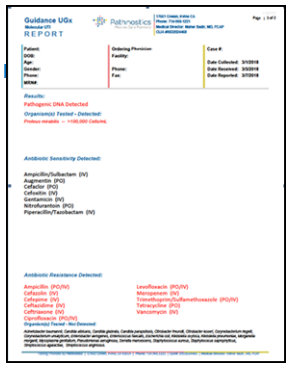
Spec plate containing antibiotics is inoculated with sample/growth media mixture. If gram-negative organism is detected, sample is also plated on CHROMagar for ESBL testing.

Spec plate is incubated for 12-16 hours. CHROMagar is incubated for 12-18 hours.

Spec plate is loaded into the plate reader and the OD measurements for each well are taken.

Antibiotics	Sample 1	Sample 2	Sample 3
Ampicillin	Resistant	Resistant	Resistant
Ampicillin/Sulbactam	Sensitive	Resistant	Sensitive
Augmentin	Sensitive	Resistant	Sensitive
Cefaclor	Resistant	Resistant	Resistant
Cefazolin	Resistant	Resistant	Resistant
Cefepime	Resistant	Resistant	Sensitive
Cefoxitin	Resistant	Resistant	Sensitive
Ceftazidime	Resistant	Resistant	Sensitive
Ceftriaxone	Resistant	Resistant	Resistant
Ciprofloxacin	Sensitive	Sensitive	Resistant
Gentamicin	Sensitive	Sensitive	Resistant
Levofloxacin	Sensitive	Sensitive	Resistant
Meropenem	Resistant	Sensitive	Sensitive
Nitrofurantoin	Sensitive	Sensitive	Sensitive
Piperacillin/Tazobactam	Sensitive	Sensitive	Sensitive
Tetracycline	Resistant	Resistant	Resistant
Trimethoprim/Sulfamethoxazole	Resistant	Sensitive	Resistant
Vancomycin	Resistant	Resistant	Resistant
QC Analysis>>>	PASS	PASS	PASS
QC Detected Resistance>>>	PASS	PASS	PASS

Results are analyzed by CLS to determine sensitivity or resistance to antibiotics present in designated wells.



PATHNOSTICS
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GUIDANCE UTI

Questions. Please call 714-966-1221 or visit pathnostics.com

PATHNOSTICS GUIDANCE*COMPREHENSIVE: RECURRENT, PERSISTENT, OR COMPLICATED UTI REPORT + TB/STI

Patient: First Last DOB: 10-16-1959 Case#: PUXR19-010846-TS

POOLED PHENOTYPIC SENSITIVITY DETECTED ($):

- Ciprofloxacin (PO/IV)
- Levofloxacin (PO/IV)
- Nitrofurantoin (PO)
- Piperacillin/Tazobactam (IV)
- Tetracycline (PO)

POOLED PHENOTYPIC RESISTANCE DETECTED (R):

- Ampicillin (PO/IV)
- Ampicillin/Sulbactam (IV)
- Cefaclor (PO)
- Cefazolin (IV)
- Cefepime (IV)
- Cefoxitin (IV)
- Ceftazidime (IV)
- Ceftriaxone (IM/IV)
- Fosfomycin (PO)
- Gentamicin (IM/IV)
- Meropenem (IV)
- Sulfamethoxazole/Trimethoprim (PO/IV)
- Vancomycin (IV)

RESISTANCE GENE(S) DETECTED (RGD):

- Ampicillin Resistance
- ESBL Resistance***
- Macrolide Resistance
- Methicillin Resistance
- Vancomycin Resistance

RESISTANCE GENE(S) TESTED - NOT DETECTED:

- Broad Spectrum*
- Carbapenem Resistance
- Quino/Fluoroquinolone

ORGANISM(S) TESTED - NOT DETECTED:

BACTERIA:

- *Acinetobacter baumannii*
- *Aerococcus urinae*
- *Citrobacter koseri*
- *Coagulase Negative Staph Group* **
- *Corynebacterium riegelii*
- *Klebsiella aerogenes*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Morganella morganii*
- *Mycobacterium tuberculosis*
- *Mycoplasma genitalium*
- *Mycoplasma hominis*
- *Pantoea agglomerans*
- *Proteus mirabilis*
- *Providencia stuartii*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Streptococcus pyogenes*
- *Ureaplasma urealyticum*

VIRUS:

- Adenovirus
- BK Virus
- Human Herpes Virus-1,-2 (HSV -1,-2)
- Human Herpes Virus-5 (CMV)
- Human Herpes Virus-6 (HHV-6)
- Human Herpes Virus-7 (HHV-7)
- JC Virus

STI:

- *Chlamydia trachomatis*
- *Neisseria gonorrhoeae*
- *Trichomonas vaginalis*

YEAST:

- *Candida albicans*
- *Candida glabrata*
- *Candida parapsilosis*

References:

* *Broad Spectrum Resistant genes identified confers resistance across multiple classes of antibiotics including penicillin derivatives, cephalosporins, monobactams and carbapenems*

** *Coagulase Negative Staphylococcus includes: Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunensis, and Staphylococcus saprophyticus*

*** *ESBL Positive for extended-spectrum beta-lactamases (ESBL) which are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. Infections with ESBL producing organisms have been associated with poor outcomes*

**** *Viridans Group Strep includes: Streptococcus anginosus, Streptococcus oralis, and Streptococcus pasteurianus*

Disclaimer: This test was developed and its performance characteristics determined by Pathnostics. It has not been cleared or approved by the US Food and Drug Administration. The FDA has determined that such clearance or approvals is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical testing. Urine specimens received greater than 5 days post collection may give unreliable cells/mL counts due to overgrowth of microorganism(s). Methodology and Clinical Significance: Microbes and resistance genes are detected through multiplex PCR. Pathogens are reported as: 'no pathogenic DNA detected' Detected: "<10,000", "10,000-99,999", "100,000-999,999", or "1M0,000" organism(s) per milliliter of urine. Resistance genes are either detected or not detected. Minimum inhibitory concentration (MIC)is determined by subjecting the polymicrobial population to a panel of antimicrobial agents. For full methodology visit pathnostics.com/methodology V1.09-1

Electronically Signed By: Bryan Chow, CLS, CGMBS, MB (ASCP) at 01/29/2020 09:31 am

GUIDANCE UTI

Questions. Please call 714-966-1221 or visit pathnostics.com

PATHNOSTICS GUIDANCE*COMPREHENSIVE: RECURRENT, PERSISTENT, OR COMPLICATED UTI REPORT + TB/STI

Patient: First Last

DOB: 10-16-1959
Gender: F
Phone:
MRN#:

Physician: Doctor
Facility: Facility
Phone:
Fax:

Case#: PUXR19-010846-TS
Collection Method: Voided
Date Collected: 06-25-2019
Date Received: 06-26-2019
Date Reported: 01-29-2020

RESULTS: PATHOGENIC DNA DETECTED

ORGANISM(S) TESTED - DETECTED: (See last page for Organism(s) Tested - Not Detected)

- ***Citrobacter freundii* >100,000 cells/mL**
- ***Enterococcus faecalis* >100,000 cells/mL**
- ***Escherichia coli* >100,000 cells/mL**
- ***Viridans Group Strep* >100,000 cells/mL**
- ***Actinotignum schaalii* 50,000-99,999 cells/mL**
- ***Alloscardovia omnicolens* 50,000-99,999 cells/mL**

LEGEND	Levofloxacin	Tetracycline	Ciprofloxacin	Nitrofurantoin	Piperacillin / Tazobactam	Fosfomycin	Sulfamethoxazole / Trimethoprim	Gentamicin	Meropenem	Ampicillin	Cefaclor	Ceftriaxone	Ceftazidime	Vancomycin	Ampicillin / Sulbactam	Cefepime	Cefazolin	Cefoxitin
$ = Pooled Phenotypic Sensitivity Detected R = Pooled Phenotypic Resistance Detected RGD = Resistance Gene(s) Detected																		
Formulations	PO/IV	PO	PO/IV	PO	IV	PO	PO/IV	IM/IV	IV	PO/IV	PO	IM/IV	IV	IV	IV	IV	IV	IV
Pooled Phenotypic Sensitivity	$	$	$	$	$	R	R	R	R	R	R	R	R	R	R	R	R	R
Resistance Gene(s) Detected					RGD					RGD	RGD	RGD	RGD	RGD	RGD	RGD	RGD	RGD
MIC Results (ug/mL)	1	2	1	32	16/4													

Organism(s) Tested - Detected: ✓ = Check marks are supportive data and are NOT patient specific.

Citrobacter freundii	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓			✓		
Enterococcus faecalis	✓	✓	✓	✓	✓	✓			✓	✓				✓	✓			
Escherichia coli	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Viridans Group Strep ****	✓	✓			✓				✓	✓		✓	✓	✓	✓	✓	✓	
Actinotignum schaalii	✓	✓	✓	✓				✓		✓		✓		✓				
Alloscardovia omnicolens	✓	✓	✓	✓	✓	✓		✓		✓			✓	✓				

✓ = Check marks indicate situations for which antibiotic use is either FDA-approved or off label use for antibiotics is illustrated in peer review literature. References available upon request.

RESULTS: PATHOGENIC DNA DETECTED

ORGANISM(S) TESTED - DETECTED: (See last page for Organism(s) Tested - Not Detected)

- *Citrobacter freundii >100,000 cells/mL*
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- *Viridans Group Strep >100,000 cells/mL*
- *Actinotignum schaalii 50,000-99,999 cells/mL*
- *Alloscardovia omnicolens 50,000-99,999 cells/mL*

LEGEND

S = Pooled Phenotypic Sensitivity Detected

R = Pooled Phenotypic Resistance Detected

RGD = Resistance Gene(s) Detected

	Levofloxacin	Tetracycline	Ciprofloxacin	Nitrofurantoin	Piperacillin / Tazobactam	Fosfomycin	Sulfamethoxazole / Trimethoprim	Gentamicin	Meropenem	Ampicillin	Cefaclor	Ceftriaxone	Ceftazidime	Vancomycin	Ampicillin / Sulbactam	Cefepime	Cefazolin	Cefoxitin
Formulations	PO/IV	PO	PO/IV	PO	IV	PO	PO/IV	IM/IV	IV	PO/IV	PO	IM/IV	IV	IV	IV	IV	IV	IV
Pooled Phenotypic Sensitivity	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R
Resistance Gene(s) Detected					RGD					RGD	RGD	RGD	RGD	RGD	RGD	RGD	RGD	RGD
MIC Results (ug/mL)	1	2	1	32	16/4													

Organism(s) Tested - Detected: ✓ = Check marks are supportive data and are NOT patient specific.

Citrobacter freundii	✓	✓	✓	✓	✓		✓	✓	✓			✓	✓			✓		
Enterococcus faecalis	✓	✓	✓	✓	✓	✓			✓	✓				✓	✓			
Escherichia coli	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Viridans Group Strep ****	✓	✓			✓				✓	✓		✓	✓	✓	✓	✓	✓	
Actinotignum schaalii	✓	✓	✓	✓				✓		✓		✓		✓				
Alloscardovia omnicolens	✓	✓	✓	✓	✓	✓		✓		✓			✓	✓				

✓ = Check marks indicate situations for which antibiotic use is either FDA-approved or off label use for antibiotics is illustrated in peer review literature. References available upon request.

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Clinical Trials Results

Current Clinical Studies

Studies	Internal Validation	Retrospective UTI Urology Study (2018-RUUS)	Prospective UTI Urology Study (2018-PUUS)	Retrospective VPA Home Care UTI Study (2019 R-VPA-HUS)
Purpose	Technical Validity	Clinical Validity	Clinical Utility	Clinical Utility
Cohort Size	NA	500	2,518	110,000
Population	NA	60 and older	Mean age of 73	Mean age 70
Sites	Internal Test Validation	Urology Clinic (1)	37 Urology Offices in 7 states with 75 physicians	Home Care Patients
Study Design	NA	Retrospective analysis comparing Guidance UTI to culture	Compare outcomes of patients treated based upon Culture or Guidance UTI results	Comparison of culture to Guidance UTI in diagnosing and managing patients with UTI
Principal Investigator	Michael Opel, PhD	Kirk Wojno, MD	Kirk Wojno, MD	Kirk Wojno, MD
Status	Completed	Completed	Paper Submitted	Paper Submitted

Bacterial Detection

	Guidance Positive n (%)	Guidance Negative n (%)	Total n (%)	Agreement n (%)	p-value
Culture Positive	1,018 (40.5%)	80 (3.2%)	1,098 (43.7%)	92.7% (91.4,94.1%)	<0.001
Culture Negative	557 (22.2%)	856 (34.1%)	1,413 (56.3%)	60.6% (58.0,63.1%)	
Total	1,575 (62.7%)	936 (37.3%)	2,511 (100.0%)	74.6% (72.9,76.3%)	

2018-PUUS Western IRB Number: 20181661 Not yet published.

Compare Ability to Detect Polymicrobial Infections

	Guidance Polymicrobial (≥2 bacteria)	Guidance Positive (Monomicrobial)	Guidance Negative	Total	Agreement
Culture Polymicrobial (≥2 bacteria)	**141** (5.6%)	**25** (1.0%)	**2** (0.1%)	**168** (6.7%)	83.9%
Culture Positive (Monomicrobial)	**425** (16.9%)	**427** (17.0%)	**78** (3.1%)	**930** (37.0%)	45.9%
Culture Negative	**268** (10.7%)	**289** (11.5%)	**856** (34.1%)	**1,413** (56.3%)	60.6%
Total	**834** (33.2%)	**741** (29.5%)	**936** (37.3%)	**2,511** (100.0%)	56.7%

2018-PUUS Western IRB Number: 20181661 Not yet published.

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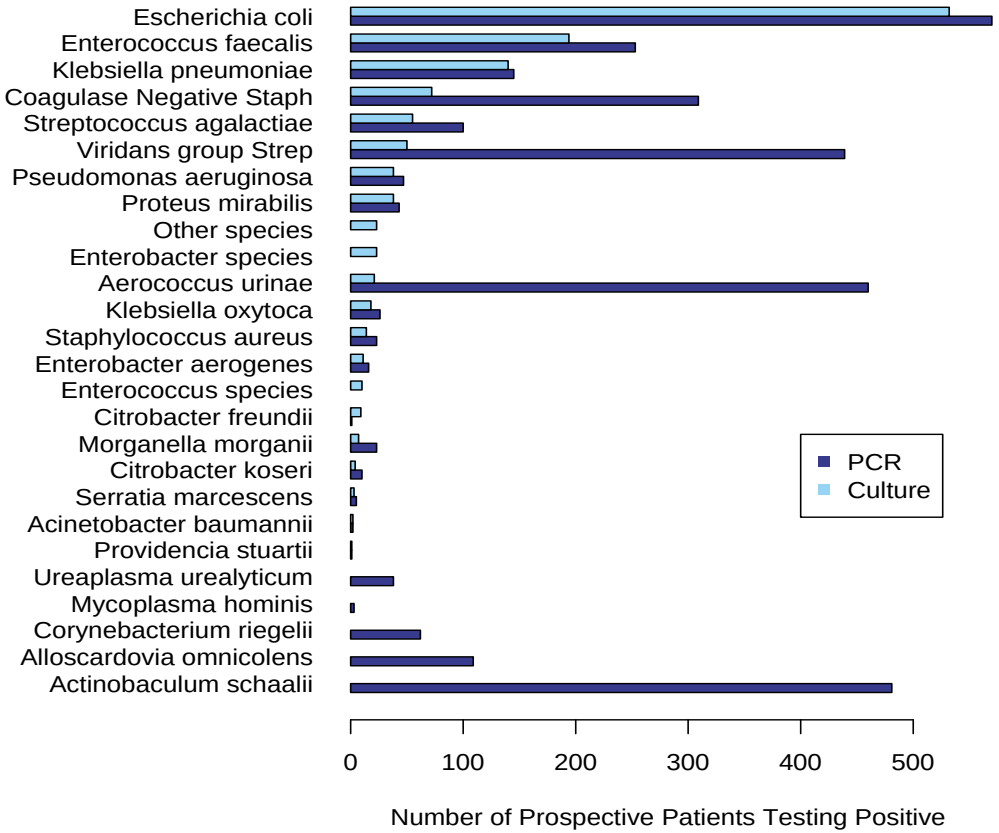
Bacterial Histogram-Prospective Study



Identification of Organisms

- Uses Real Time PCR to detect organisms associated with Urinary Tract Infections
- Quantity of Identified Organisms
  - Between 1,620 and 5,401 cells/mL (depending on organism) to 6,000,000 cells/mL or greater

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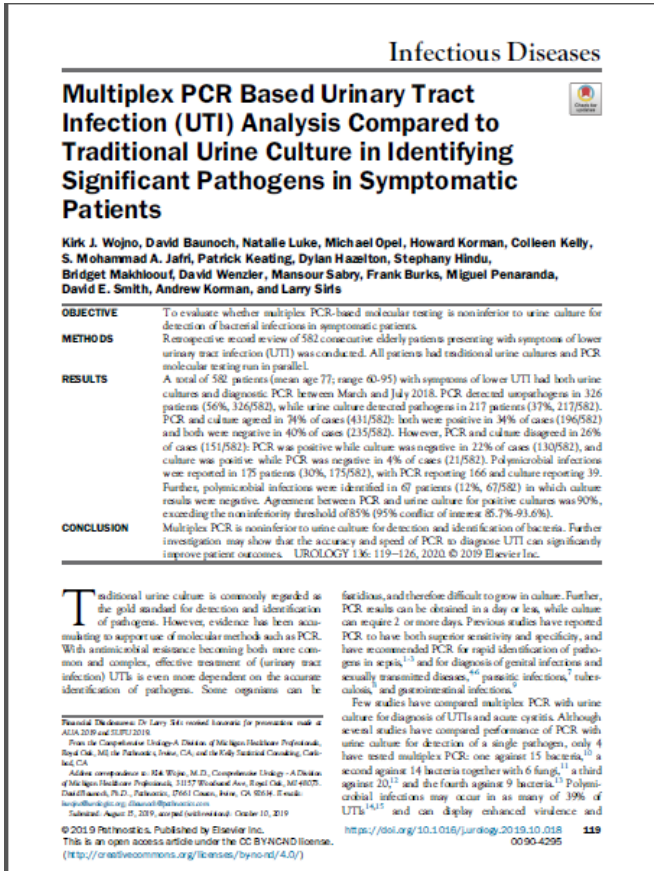
Western IRB Number: 20181661

Retrospective Paper – In Press



Infectious Diseases

Multiplex PCR Based Urinary Tract Infection (UTI) Analysis Compared to Traditional Urine Culture in Identifying Significant Pathogens in Symptomatic Patients

Kirk J. Wojno, David Baunoch, Natalie Luke, Michael Opel, Howard Korman, Colleen Kelly, S. Mohammad A. Jafri, Patrick Keating, Dylan Hazelton, Stephany Hindu, Bridget Makhlouf, David Wenzler, Mansour Sabry, Frank Burks, Miguel Penaranda, David E. Smith, Andrew Korman, and Larry Sirls

OBJECTIVE To evaluate whether multiplex PCR-based molecular testing is noninferior to urine culture for detection of bacterial infections in symptomatic patients.

METHODS Retrospective record review of 582 consecutive elderly patients presenting with symptoms of lower urinary tract infection (UTI) was conducted. All patients had traditional urine cultures and PCR molecular testing run in parallel.

RESULTS A total of 582 patients (mean age 77; range 60-95) with symptoms of lower UTI had both urine cultures and diagnostic PCR between March and July 2018. PCR detected uropathogens in 326 patients (56%, 326/582), while urine culture detected pathogens in 217 patients (37%, 217/582). PCR and culture agreed in 74% of cases (431/582): both were positive in 34% of cases (196/582) and both were negative in 40% of cases (235/582). However, PCR and culture disagreed in 26% of cases (151/582): PCR was positive while culture was negative in 22% of cases (130/582), and culture was positive while PCR was negative in 4% of cases (21/582). Polymicrobial infections were reported in 175 patients (30%, 175/582), with PCR reporting 166 and culture reporting 39. Further, polymicrobial infections were identified in 67 patients (12%, 67/582) in which culture results were negative. Agreement between PCR and urine culture for positive cultures was 90%, exceeding the noninferiority threshold of 85% (95% confict of interest 85.7%-93.6%).

CONCLUSION Multiplex PCR is noninferior to urine culture for detection and identification of bacteria. Further investigation may show that the accuracy and speed of PCR to diagnose UTI can significantly improve patient outcomes. UROLOGY 136: 119–126, 2020. © 2019 Elsevier Inc.

Traditional urine culture is commonly regarded as the gold standard for detection and identification of pathogens. However, evidence has been accumulating to support use of molecular methods such as PCR. With antimicrobial resistance becoming both more common and complex, effective treatment of (urinary tract infection) UTIs is even more dependent on the accurate identification of pathogens. Some organisms can be fastidious, and therefore difficult to grow in culture. Further, PCR results can be obtained in a day or less, while culture can require 2 or more days. Previous studies have reported PCR to have both superior sensitivity and specificity, and have recommended PCR for rapid identification of pathogens in sepsis,[1-3] and for diagnosis of genital infections and sexually transmitted diseases,[4-6] parasitic infections,[7] tuberculosis,[8] and gastrointestinal infections.[9]

Few studies have compared multiplex PCR with urine culture for diagnosis of UTIs and acute cystitis. Although several studies have compared performance of PCR with urine culture for detection of a single pathogen, only 4 have tested multiplex PCR: one against 15 bacteria,[10] a second against 14 bacteria together with 6 fungi,[11] a third against 20,[12] and the fourth against 9 bacteria.[13] Polymicrobial infections may occur in as many of 39% of UTIs[14,15] and can display enhanced virulence and

Financial Disclosures: Dr Larry Sirls received honoraria for presentations made at AUA 2018 and SUFU 2019.

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Submitted: August 15, 2019, accepted (with revisions): October 10, 2019

https://doi.org/10.1016/j.urology.2019.10.018 119
0090-4295

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Genotype v Phenotype

Resistance Gene and Susceptibility Agreement

ABR and Genotype Agree		ABR and Genotype Disagree	
Bacteria Sensitive & No R. Genes	Bacteria Resistant & R. Genes Present	Bacteria Resistant & R. Genes Not Present	Bacteria Sensitive & R. Genes Present
42%	16%	24%*	18%**
58%		42%	

*There are either more genes to detect or organism interactions impacting resistance patterns

**Genes may be present in low quantity, inactive, or nonfunctional

Bacteria Interactions

Organisms

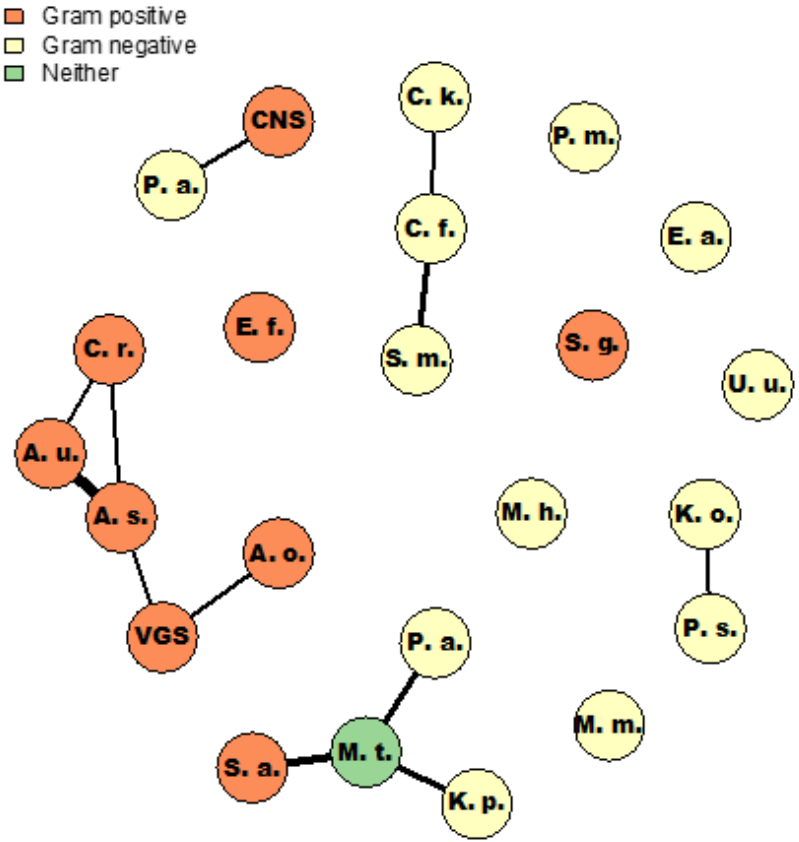
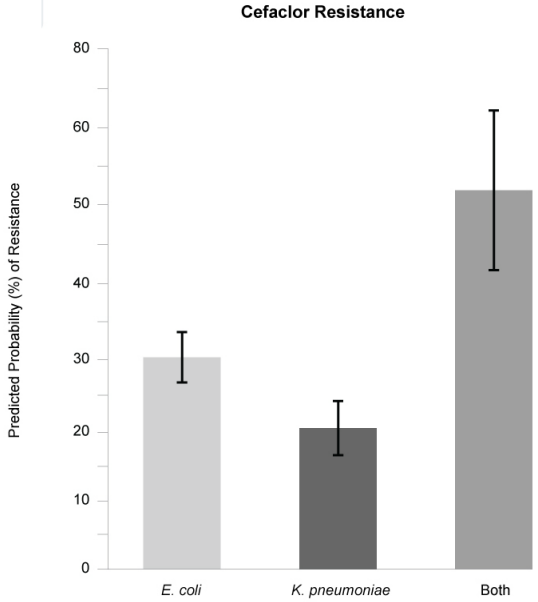
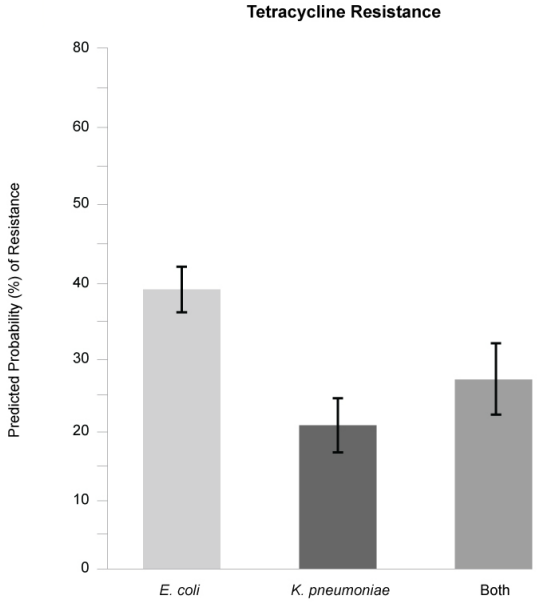


Figure 5: Correlations between organisms, excluding E. coli. The strength of the correlation is represented by the width of the edge connecting the genes. Only correlations greater than 0.1 shown.

Organism Interactions Impact Susceptibility Results



Pair increases resistance.



Would expect the antibiotic resistance levels to equal *E. coli*. *K. pneumonia* causes resistance to drop.

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Organism Interactions: Cephalosporin

Antibiotic	*Bacteria that increase odds of resistance (odds-ratio)*	*Bacteria that decrease odds of resistance (odds-ratio)*	*Interactions*
Cefaclor	E. faecalis ▲ (2.3) Pseudomonas aeruginosa (8.6)	Klebsiella pneumonia (0.69)	• E. coli & E. faecalis and Klebsiella & E. faecalis together **decrease** the odds of resistance • E. coli & Klebsiella together **increase** the odds of resistance
Cefazolin	Actinobaculum schaalli (1.3) Pseudomonas aeruginosa (2.2)		• Pseudomonas & E. faecalis together decrease the odds of resistance • E. coli & Klebsiella together increase the odds of resistance
Cefepime	E. faecalis ▲ (9.4) E. coli (1.7)		• E. coli & Klebsiella and E. coli & Proteus together increase the odds of resistance
Cefoxitin			• Pseudomonas & E. faecalis together decrease the odds of resistance • E. coli & Klebsiella together increase the odds of resistance
Ceftazidime	E. faecalis ▲ (7.3)	Klebsiella pneumonia (0.53)	• CNS & Klebsiella together decrease the odds of resistance • E. coli & Klebsiella and E. coli & Pseudomonas together increase the odds of resistance
Ceftriaxone	E. faecalis ▲ (6.9)	Klebsiella pneumonia (0.65) Proteus mirabilis (0.41) VGS (0.73)	• Pseudomonas & CNS together decrease the odds of resistance • E. coli & Klebsiella together increase the odds of resistance

▲ Even though agent is found susceptible in vitro, it is ineffective clinically

Conclusions

- Multiplex PCR for UTI demonstrates
  - improved detection of organisms in urine
  - improved detection of polymicrobial urinary tract infections
- Pooled Antibiotic Sensitivity and identification of Antibiotic Resistance Genes may better clinical utility than culture
  - Reduces the need for empiric therapy with quicker turn around time
  - Identifies effective antibiotics for the patient's specific group of organisms causing the UTI which improves time to symptom resolution

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