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Reducing Time to Result for Urinary Tract Pathogen Detection Utilizing Real-Time PCR Technology

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Evolving Picture of Urinary Tract Infections

- The Scope of the Problem
- Evaluating the Tools Available
- Refining the Definition
- Moving from a Monocentric Thought Process
- Understanding the Prevalence and Nature of Polymicrobial Infections
- Development of New Tools for the Diagnosis and Management of Urinary Tract Infections

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Objectives

- At the conclusion of the presentations participants will be able to :
  - Understand the Impact of UTI's in Patient Care
  - Evaluate the Technical Challenges Associated with Urine Culture
  - Identify the Different Classes of Uropathogens
  - Explain the Role of the Urinary Microbiome and Polymicrobial Infections in the Management of Urinary Tract Infections.
  - Assess the Use of qPCR in the Diagnosis of Urinary Tract Infections
  - Compare the Clinical Utility of Genotypic and Phenotypic Methods in Treatment of Urinary Tract Infections

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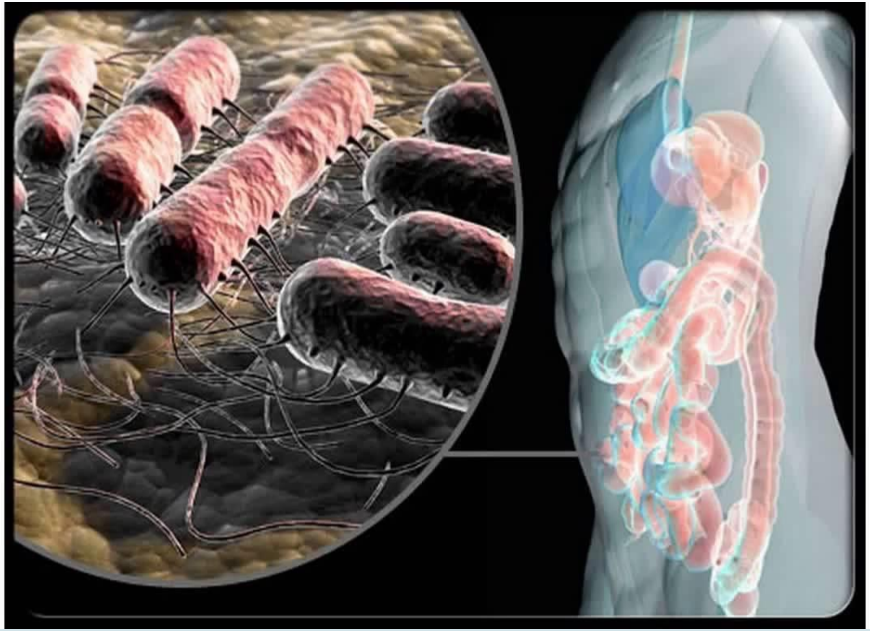
THE SCOPE OF THE PROBLEM
MARKET SIZE AND INCIDENCE

Symptoms of Urinary Tract Infections

- Pressure in Lower Pelvis
- Dysuria
- Frequency
- Urgency
- Nocturia
- Abnormal Color or Odor
- Hematuria
- Flank Pain
- Fever/Chills
- Mental Changes/Confusion



The Impact

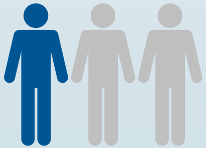
- Cost to System

Responsible for
≈10.5
MILLION
office visits/year[1]

UTI complications result in
9-11
DAYS
longer for each hospital stay[2]

Health care cost exceed
≈$13
BILLION
in the US[1]

- 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]

UTIs Segmentation (US)

Urinary Tract Infections (UTIs)

- **Symptomatic** ~12M
  - *~10-15% of adult women/year[1,2]; 2-3M ER visits (2013)[3]*
  - **Uncomplicated UTIs** (Low risk)
  - **Complicated UTIs** (High risk)
    - Cystitis — *Majority are uncomplicated[4]*
    - Pyelonephritis — *Majority are uncomplicated[4]*
    - Recurrent UTI ~3M — *25% of UTIs in women will recur within 6 months of initial infection[1]*
    - Catheter-associated UTI 1M — *Incidence: 449,334 CAUTI events (2002)[5]; 35,600 CAUTI events (2011)[6] in acute care hospitals*
    - UTI in men ~1.1M — *Incidence: 0.1% in 30-65 years old; 5% in 65-85 years old[2]*
    - Urosepsis 400K
- **Asymptomatic**
  - Pregnant women ~4M — *Asymptomatic bacteriuria (ABU): 2-10% of pregnant women[10]*
  - Patient who will undergo invasive urologic procedure (eg, prostate biopsy) ~1M
  - Renal transplant recipient
  - Patient with urinary catheter

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US Market

Total Cost of Long Term Care Derived UTI – $647M

- Long Term Care Facilities
  - 1.5M under care in 16,700 Nursing Homes with a total of 5.3M forecasted for 2030
    - Up1.2 Million infections per year with patients averaging 3-4 courses of antibiotics annually
    - Up to 120,000 Hospital admissions annually which make up 30-50% of all Medicare Hospital admissions annually
- Average Cost of Treating UTI in Nursing Home of $150 Per Patient
  - Total Cost of $180M
- Up to 120,000 Hospitalization
  - from Average Hospital stay per admission costs an average of $1947 per night
- Total Cost of Hospital Admission from Long Term Care Facilities - $467M

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US Market

Total Cost of Hospital Acquired UTI $2.2 Billion

- Hospital Acquired Urinary Tract Infections
  - 25% of patients in the US have catheters.
    - Catheter-acquired urinary infection is the source for about 20% of episodes of health-care acquired bacteremia in acute care facilities, and over 50% in long-term care facilities
  - 561,667 infections per year with a patient
  - Average 2 additional nights based on UTI
- Average Hospital stay per admission costs an average of $1947 per night

Type	Number	Cost per Night
State/Local	1053	$1,974.00
Non-Profit	1003	$2,346.00
For Profit	2870	$1,798.00
	4926	$1,947

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Pediatric Urinary Tract Infections

- 3% of children per year develop a urinary tract infection accounting for 1 million officer visits per year
- Recurrent infections occur in up to 50% of patients
- Permanent renal cortical scarring may occur in up to 65% of affected children, especially in recurrent UTI and its long-term complications include hypertension and chronic renal failure which may result in end stage renal disease
- 1.5 million office visits annually
  - $150M in annual costs
- >50,000 hospital admissions
  - Average cost of hospitalization is $10,489 per patient
  - Annual cost of $520 Million
- Total cost >$670 Million
  - Does not include costs associated with treatment of patients for consequences of renal/cortical scarring

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Sepsis

- 30 million sepsis cases worldwide annually
- 1.1 million cases in US annually
- Urosepsis comprises 25% of that total
- Total US cost for sepsis treatment is $24B annually
- Total cost for sepsis due to urinary tract infection is >$6B annually

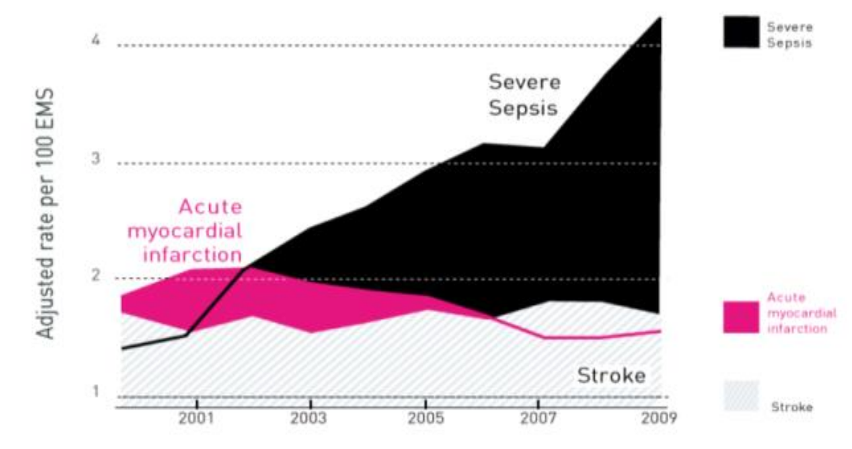

Fig 1. hospital admissions for sepsis have overtaken those for stroke or myocardial infarction. Adapted from Seymour et al. [16]

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Total Cost of UTI Treatment in US Healthcare System

Type	Cost
Physician Office Based	$3.9B
LTC Facility Based	$647M
HAI Based	$2.2B
Pediatric UTI	$.67B
Sepsis (UTI Based)	$6B

$13 Billion Dollars Annually

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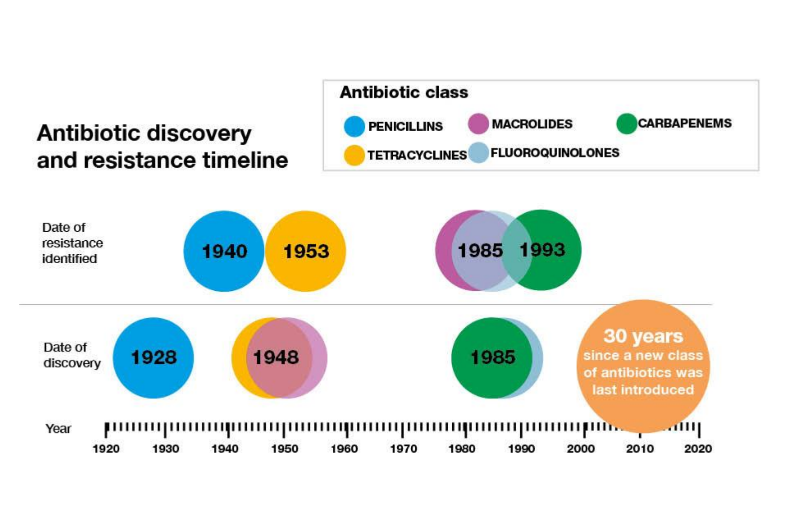
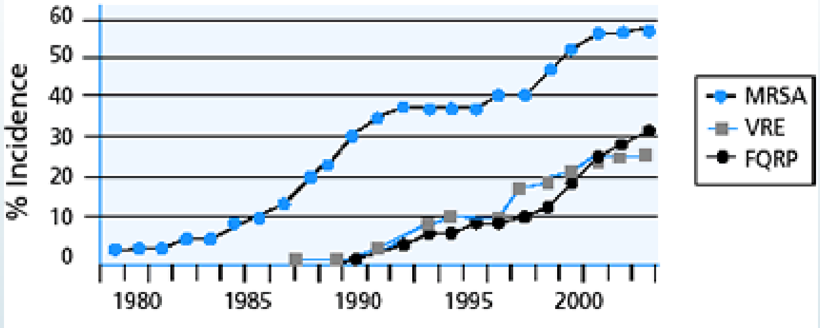
The Situation is Complicated by Lack of New Antibiotics and Increasing Rates of Antibiotic Resistance



Resistant Strains Spread Rapidly

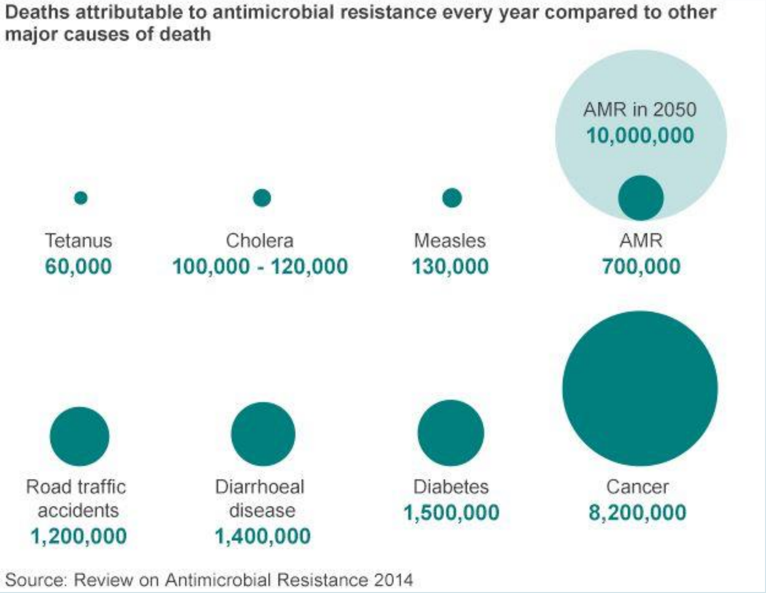


Source: Centers for Disease Control and Prevention

MRSA = Methicillin-resistant *Staphylococcus Aureus*
VRE = Vancomycin-resistantant Enterococci
FQRP = Floroquinolone-resistant *Pseudomonas aeruginosa*

Limited Efficacy of Current Testing Methodologies Limit Treatment Options

Due to Prolonged Turn around Times (48-72 Hours) and Limited Sensitivity Associated with Urine Culture Clinicians Frequently Treat Patients Empirically Resulting in Poor Antibiotic Stewardship and Increased Rate of Antibiotic Resistance.



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EVALUATING THE TOOLS

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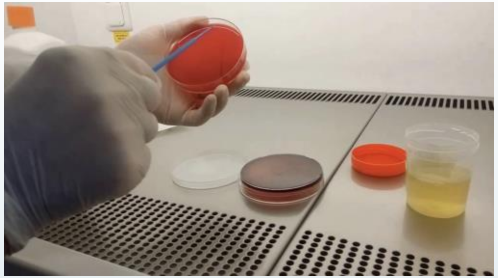
Urine Culture – The Current Gold Standard for Urinary Tract Infections

- The Method – 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.



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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*
  - The biased results developed using this methodology often creates findings that are not consistent with the clinical symptoms
  - Is unable to detect slow growing organisms including fastidious and non aerobic 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

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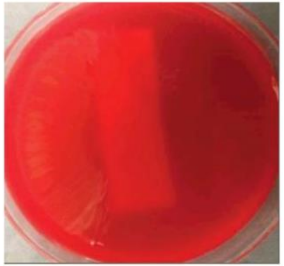
What Has Been Missed by Culture?

- The Loyola Study followed 150 patients who were split into two groups based on the whether they believed they were symptomatic for UTI
  - They 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%.

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

Price TK, et al. *J Clin Microbiol.* 2016;54[5]:1216– 1222

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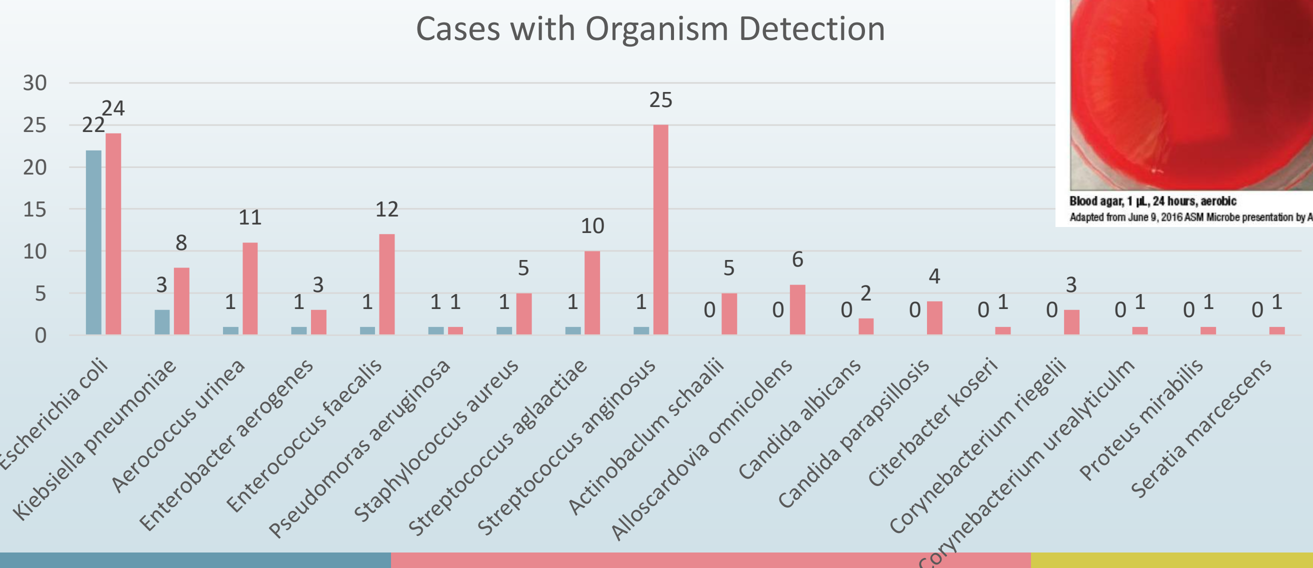
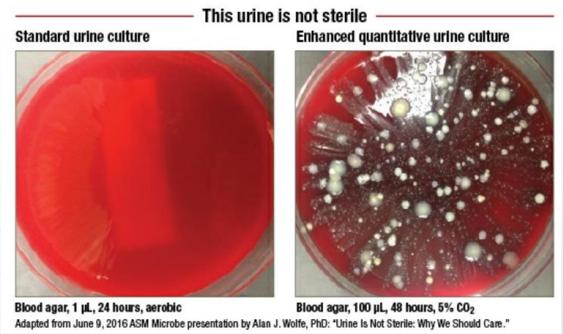
Expanding the Number and Types of Uropathogens qPCR Assay Results





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Frequency of Uropathogens - Type

	Number of Positives	Percentage
Enterococcus faecalis	252	50.4%
Escherichia coli	246	49.2%
Actinobaculum schaalii	139	27.8%
Streptococcus anginosus	133	26.6%
Morganella morganii	121	24.2%
Aerococcus urinae	114	22.8%
Klebsiella pneumoniae	92	18.4%
Proteus mirabilis	89	17.8%
Streptococcus agalactiae	53	10.6%
Alloscardovia omnicolens	43	8.6%
Candida albicans	35	7.0%
Corynebacterium riegelii	30	6.0%
Staphylococcus aureus	28	5.6%
Pseudomonas aeruginosa	26	5.2%
Klebsiella oxytoca	25	5.0%
Acinetobacter baumannii	25	5.0%

Gram+

Gram -

Common Organisms Culture Identified Positive	
Escherichia coli	Klebsiella
Enterococcus	Proteus

Fungal Uropathogens

- Fungal Infections most often due to Candida species including
  - Candida albicans
  - Candida glabrata
  - Candida parapsilosis
- Can cause both UTI's and Prostatitis
- Most patients asymptomatic but symptomatic patients are indistinguishable from those with bacterial UTI.

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Virus in the Urinary Tract

- Virus typically difficult to detect in the bladder or prostate.
- Most common viral causes of urinary tract infections include -BK virus, JC virus, Adenovirus, CMV and HSV
- Impacts those with low immunity, for example:
  - Bone marrow or organ transplantation
  - Blood cancers/malignancies (e.g.leukemia)
  - HIV infection
  - Pregnancy
  - Diabetes, alcoholism, malnutrition, liver cirrhosis
- In UTI, high viral load is associated with high mortality in patients with low immunity

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UNDERSTANDING THE IMPACT OF THE MICROBIOME AND POLYMICROBIAL INFECTIONS

REFINING THE DEFINITION – MOVING FROM A MONO-CENTRIC THOUGHT PROCESS

Evolving Picture of the Microbiome of the Bladder and Urethra

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Evidence of Uncultivated Bacteria in the Adult Female Bladder

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Clinical urine specimens are usually considered to be sterile when they do not yield uropathogens using standard clinical cultivation procedures. Our aim was to test if the adult female bladder might contain bacteria that are not identified by these routine procedures. An additional aim was to identify and recommend the appropriate urine collection method for the study of bacterial communities in the female bladder. Consenting participants who were free of known urinary tract infection provided urine samples by voided, transurethral, and/or suprapubic collection methods. The presence of bacteria in these samples was assessed by bacterial culture, light microscopy, and 16S rRNA gene sequencing. Bacteria that are not or cannot be routinely cultivated (hereinafter called uncultivated bacteria) were common in voided urine, urine collected by transurethral catheter (TUC), and urine collected by suprapubic aspirate (SPA), regardless of whether the subjects had urinary symptoms. Voided urine samples contained mixtures of urinary and genital tract bacteria. Communities identified in parallel urine samples collected by TUC and SPA were similar. Uncultivated bacteria are clearly present in the bladders of some women. It remains unclear if these bacteria are viable and/or if their presence is relevant to idiopathic urinary tract conditions.

Compared Samples Obtained From Voided Urine, and Transurethral Catheters to Specimens Obtained by Suprapubic Aspiration – and They Were Very Similar

- Urine is not sterile
- The bladder contains a microbiome that has been overlooked primarily because of our limited capacity to culture microorganisms
- The net result has been an understatement of the frequency and scope of bacterial infections

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The Female and Male Microbiome

- The characterization of the male and female urinary microbiome are in their infancy but recent studies have begun to define the basic parameters associated with them.
    - In Females, the FUM
        - Tend to be at lower colony counts as compared to other human microbiomes with counts in the 10^3 to 10^5 range.
        - They are dominated by Lactobacillus, Gardnerella, Sneathia, Staphylococcus and Enterbacteriaceae as well as other diverse species.
        - They consist of genital and urinary tract organisms.

Table detailing the genera identified within each defined age group for females only.

All ages ($n = 23$)	Age 20–49 ($n = 13$)	Age 50–69 ($n =$
Actinobaculum	***Azospira***	*Brevibacterium*
Anaerococcus	***Butyricicoccus***	***Catonella***
Anaerosphaera	***Coriobacterium***	***Caulobacter***
Atopobium	***Friedmanniella***	***Methylovirgula***
Campylobacter	*Gardnerella*	***Pelomonas***
Corynebacterium	***Microvirgula***	*Peptostreptococ*
Dialister	*Neisseria*	***Sneathia***
Enterobacter	*Paraprevotella*	***Streptophyta***
Enterococcus	***Rhodopila***	***Thermoleophil***
Facklamia	***Sutterella***	
Finegoldia	***Tepidimonas***	
Fusobacterium	***Tessaracoccus***	
Lactobacillus	***TM7_genera_incertae_sedis***	
Mobiluncus		
Murdochiella		
Negativicoccus		
Peptoniphilus		
Porphyromonas		
Prevotella		
Propionimicrobium		
Sporanaerobacter		
Streptococcus		
Varibaculum		

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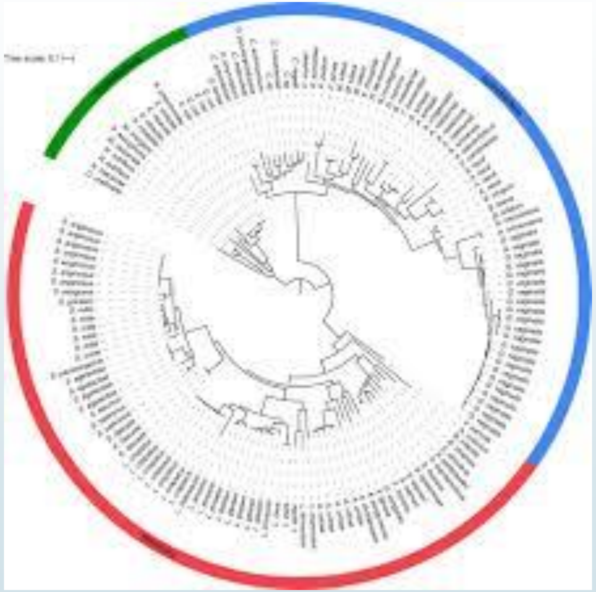
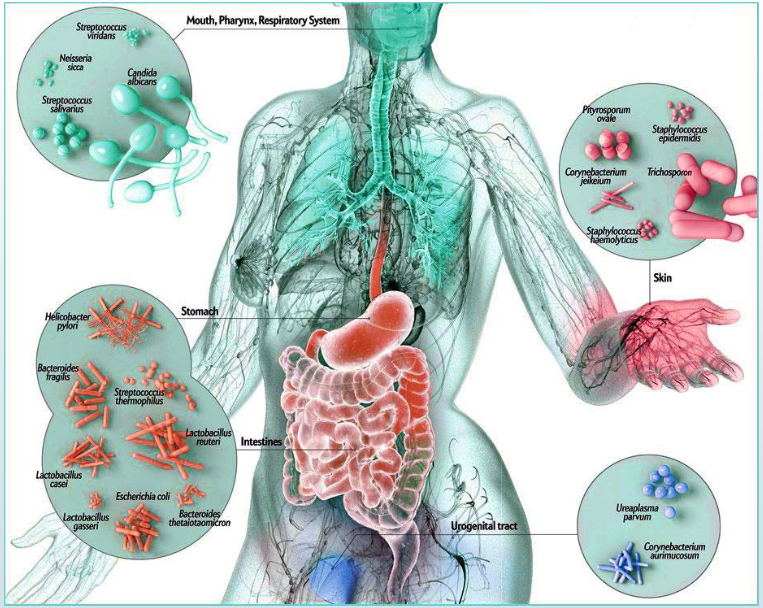
The Female and Male Microbiome

- In Males, the MUM
  - They are dominated by Lactobacillus, Sneathia, Veillonella, Corynebacterium Prevotelloa, Streptococcus, and Ureaplasma.
  - They consist of genital and urinary tract organisms.



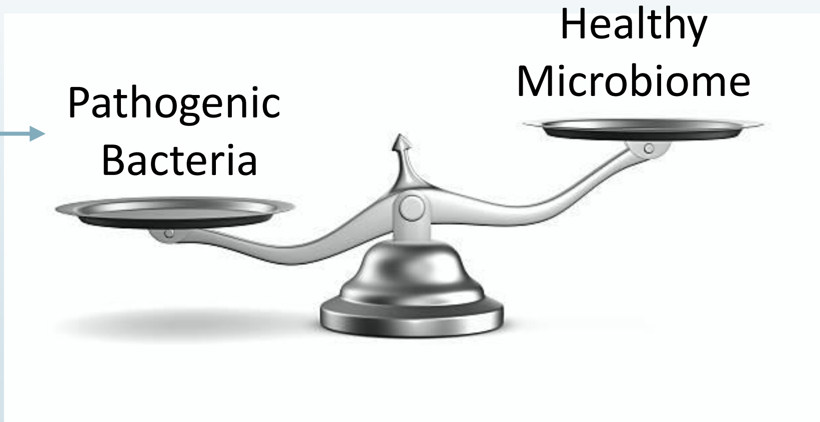
Asymptomatic Bacteriuria and Dysbiosis

- Asymptomatic bacteriuria is the presence of a high number of bacteria >100,000/ml without symptoms.
- Not treated unless patient is has renal disease, is immunocompromised or pregnant (to prevent pyelonephritis)
- May represent an ecological balance between pathogenic bacteria and the urinary microbiome.



Dysbiosis in the Urinary Tract

- Antibiotic Use
- Immune Suppression
- Diet
- Stress
- Lack of Exercise

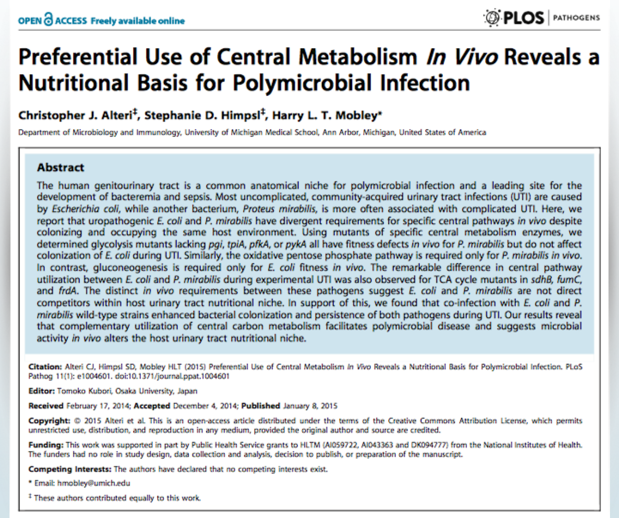


Evolving From an Monocentric View of Urinary Tract Infections

- Wolfe and Brubaker have proposed moving from a E.coli-centric view of urinary tract infections -
  - As importantly with an increasing number of studies demonstrating that the majority of 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.
    - A simple truth – Polymicrobial Infections may be the norm rather then the exception.

Bacteria Share Metabolic Products in Polymicrobial Infections

- The sharing of metabolic products provides polymicrobial infections an advantage
  - In the presence of antibiotics the sharing of metabolic products plays a protective role increasing resistance and virulence
- Brings into question the current practice of isolating organisms prior to determining the antibiotic resistance



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Preferential Use of Central Metabolism *In Vivo* Reveals a Nutritional Basis for Polymicrobial Infection

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Abstract

The human genitourinary tract is a common anatomical niche for polymicrobial infection and a leading site for the development of bacteremia and sepsis. Most uncomplicated, community-acquired urinary tract infections (UTI) are caused by *Escherichia coli*, while another bacterium, *Proteus mirabilis*, is more often associated with complicated UTI. Here, we report that uropathogenic *E. coli* and *P. mirabilis* have divergent requirements for specific central pathways *in vivo* despite colonizing and occupying the same host environment. Using mutants of specific central metabolism enzymes, we determined glycolysis mutants lacking *pgi*, *tpiA*, *pfkA*, or *pykA* all have fitness defects *in vivo* for *P. mirabilis* but do not affect colonization of *E. coli* during UTI. Similarly, the oxidative pentose phosphate pathway is required only for *P. mirabilis in vivo*. In contrast, gluconeogenesis is required only for *E. coli* fitness *in vivo*. The remarkable difference in central pathway utilization between *E. coli* and *P. mirabilis* during experimental UTI was also observed for TCA cycle mutants in *sdhB*, *fumC*, and *frdA*. The distinct *in vivo* requirements between these pathogens suggest *E. coli* and *P. mirabilis* are not direct competitors within host urinary tract nutritional niche. In support of this, we found that co-infection with *E. coli* and *P. mirabilis* wild-type strains enhanced bacterial colonization and persistence of both pathogens during UTI. Our results reveal that complementary utilization of central carbon metabolism facilitates polymicrobial disease and suggests microbial activity *in vivo* alters the host urinary tract nutritional niche.

Citation: Alteri CJ, Himpsl SD, Mobley HLT (2015) Preferential Use of Central Metabolism *In Vivo* Reveals a Nutritional Basis for Polymicrobial Infection. PLoS Pathog 11(1): e1004601. doi:10.1371/journal.ppat.1004601

Editor: Tomoko Kubori, Osaka University, Japan

Received February 17, 2014; **Accepted** December 4, 2014; **Published** January 8, 2015

Funding: This work was supported in part by Public Health Service grants to HLTM (AI059722, AI043363 and DK094777) from the National Institutes of Health. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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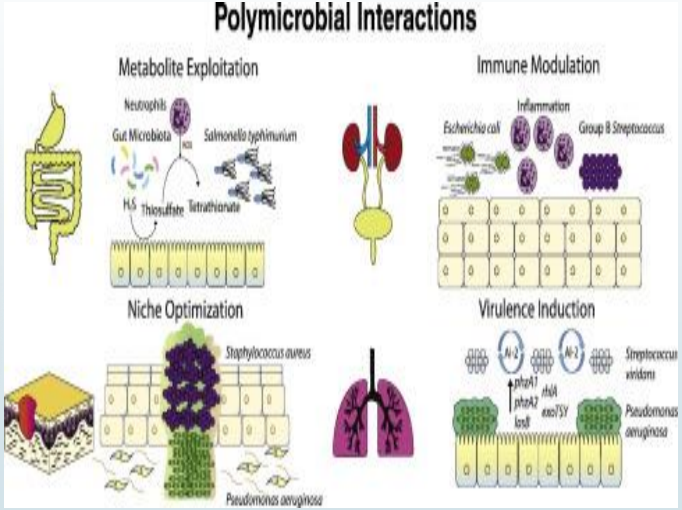

It is becoming increasingly clear that a significant number of urinary tract infections are polymicrobial in nature. Because of the polymicrobial nature of infections, efficacy of treatment is dropping significantly.

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

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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 Interactions Change MIC Levels

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]

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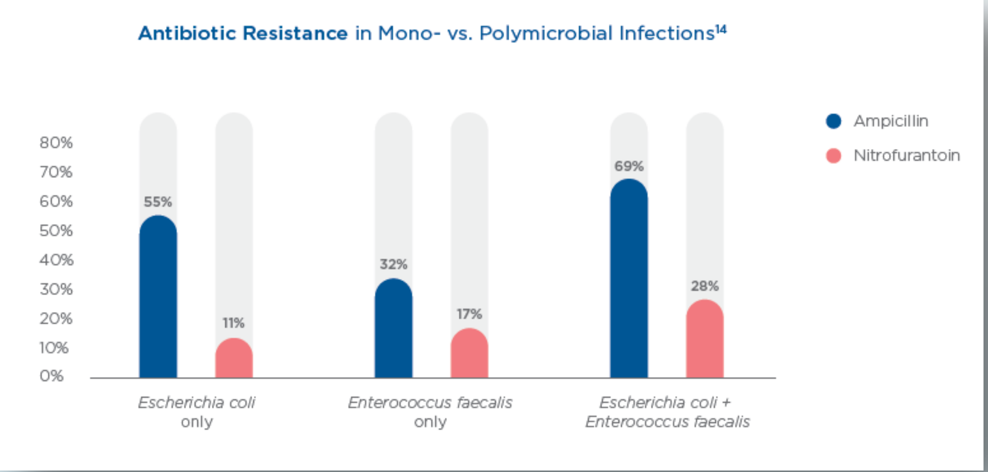
Table 1. Prevalence of antibiotic resistance in UTI isolates

'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	–	–	5.9	0	4.5	0	–	–
Cefotaxime (1)	17.8	14.29	–	–	33.9	100.0	–	–	–	–
Ceftazidime (1)	18.6	9.52	–	–	35.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Meropenem (2)	0	0	15.5	0	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Piperacillin–tazobactam (16)	6.2	4.76	4.5	25.0	23.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Co-amoxiclav (32)	5.4	0	2.7	0	16.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Trimethoprim (2)	44.2	28.57	–	–	89.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ciprofloxacin (4)	23.3	9.52	28.2	0	0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cefradine (32)	28.7	19.05	–	–	55.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Nitrofurantoin (32)	17.1	9.52	10.9	0		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Amoxicillin (32)	45.0	42.86	7.3	0	37.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Gentamicin (10)	–	–	–	–	–	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Piperacillin–tazobactam (85)	–	–	–	–	–	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ceftazidime (30)	–	–	–	–	–	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Meropenem (10)	–	–	–	–	–	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ciprofloxacin (1)	–	–	–	–	–	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]



Pathnostics

Prevalence of Polymicrobial Infections Require the Development of New Methodologies

- Using current culture guidelines polymicrobial infections would most often be classed as mixed flora—probable contamination and not be worked up…
  - In that there are a number of studies showing polymicrobial infections in the the blood with corresponding UTI findings – this supports the clinical importance of proper characterization of samples

- This lack of sensitiviy seen with traditional culture coupled with this guidance underlys the growing incidence of patients presenting with symtopms of UTI and no diagnosis—resulting in ineffective treatment

Pathnostics

DEVELOPING NEXT GENERATION TOOLS FOR THE EVALUATION OF URINARY TRACT INFECTIONS

Pathnostics

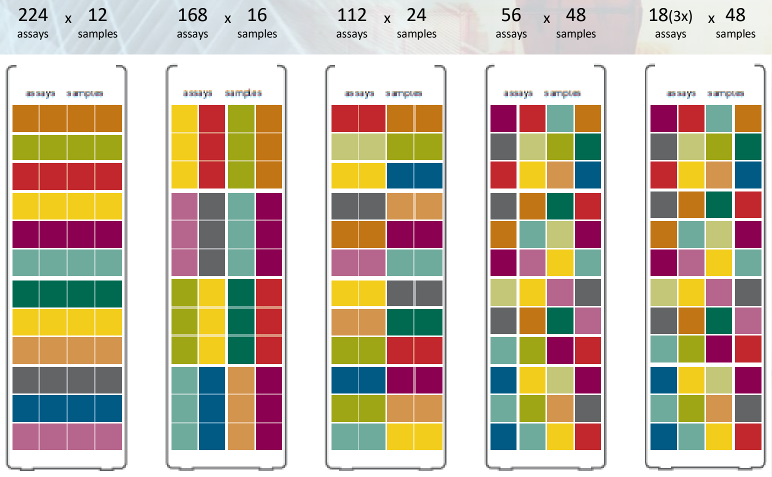
Development of a Next Generation Assay for the Identification of Urinary Tract Infections

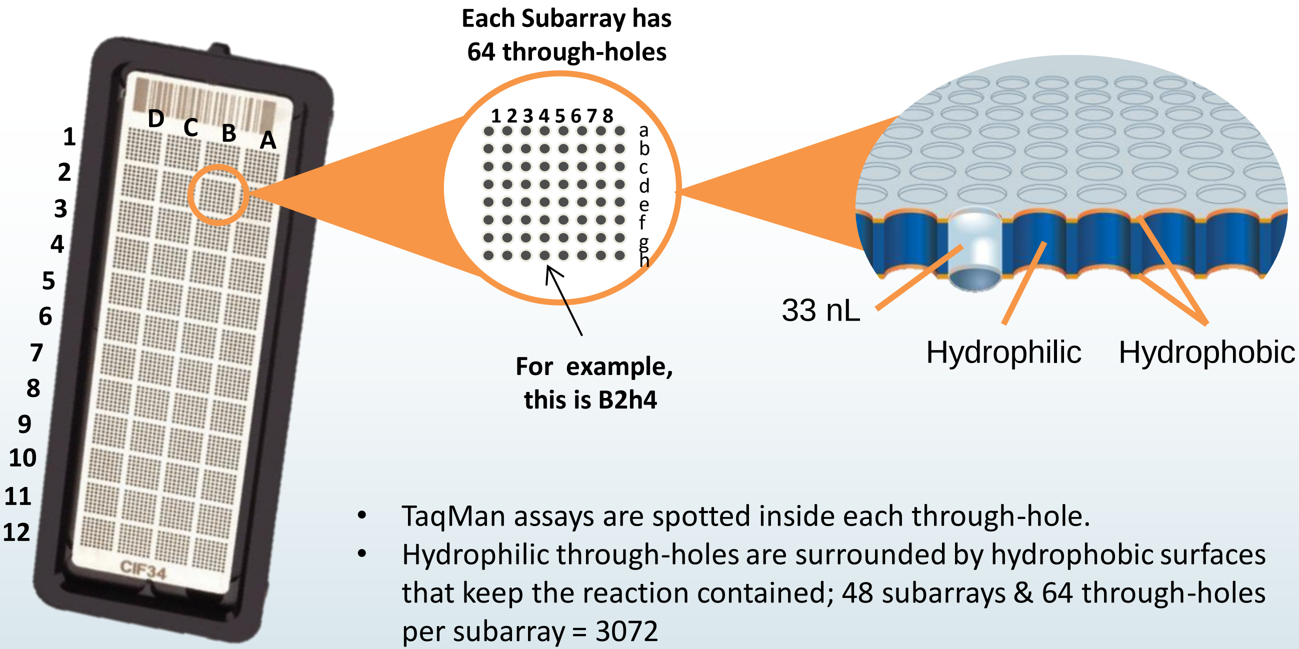
- Guidance is a quantitative PCR based assay that identifies organisms associated with UTI's without the need of culture

QuantStudio 12k Flex Real-time PCR System: For Research Use Only, not for use in diagnostic procedures.

Pathnostics

Open Array Format with 56 Assays and 48 Samples





- TaqMan assays are spotted inside each through-hole.
- Hydrophilic through-holes are surrounded by hydrophobic surfaces that keep the reaction contained; 48 subarrays & 64 through-holes per subarray = 3072

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*

VIRAL PARTICLES

- BK virus
- Adenovirus
- CMV
- HSV
- JC virus

- Sample Type
  - Urine
    - Voided, Catheter, or Suprapubic Aspiration
  - Quantity of Identified Organisms
    - Between 500 cells/mL (depending on organism) to 6,000,000 cells/mL or greater

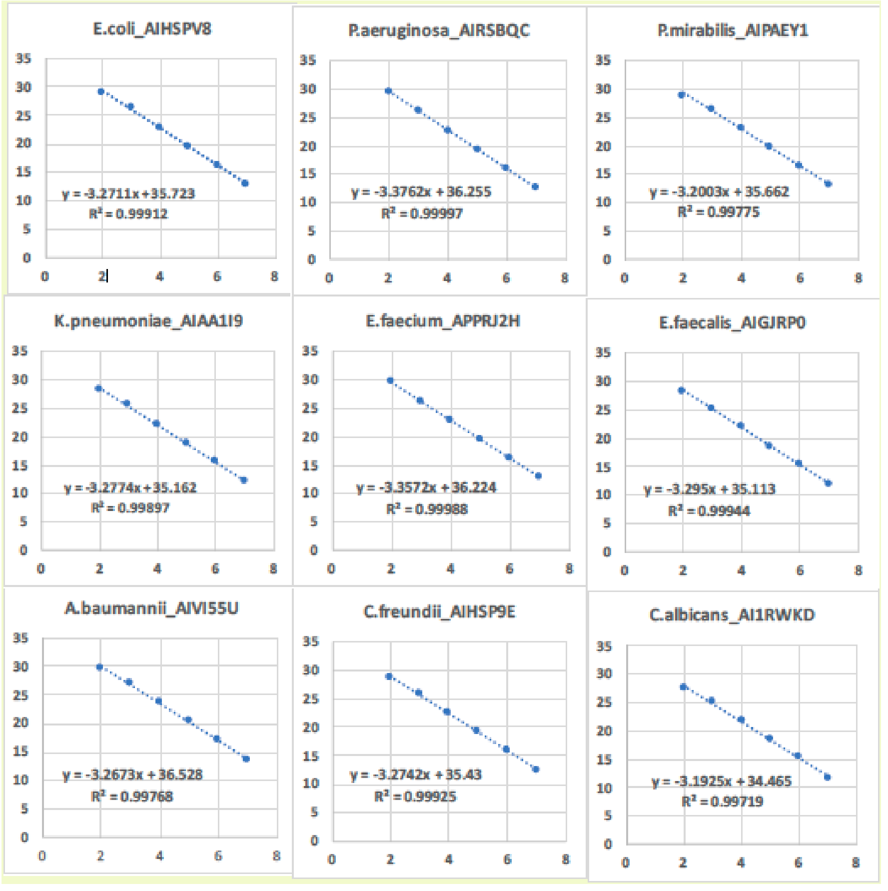
High Sensitivity and Specificity with ATCC Inclusivity Panel

on-target

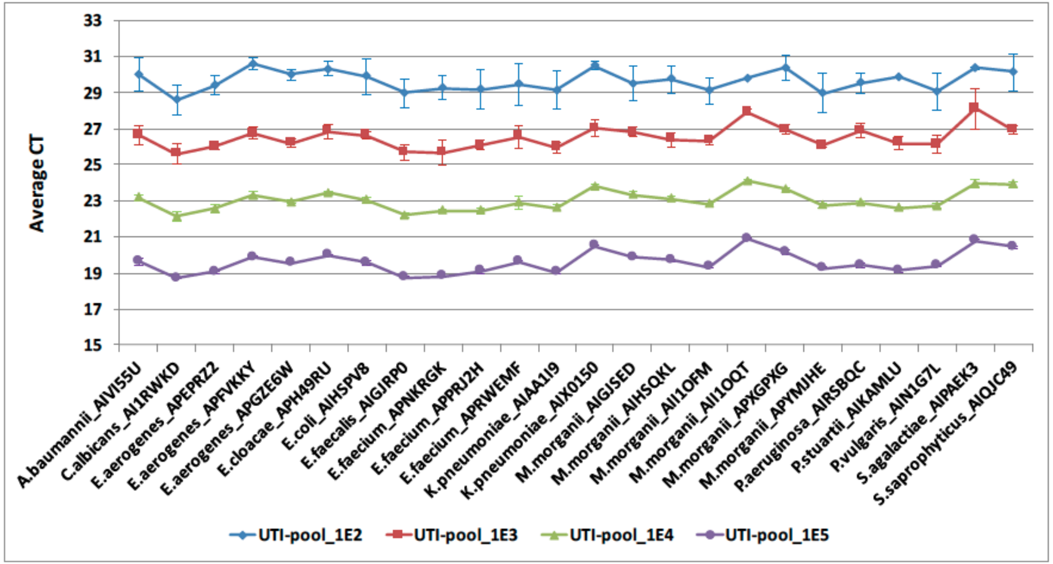
	AB	CA	CF	EA	EnC	EsC	EF_b	EF	KO	KP	MM	PA	PM	PS	PV	SA	SS	UTI-V5
A.baumannii_AIVI55U	20.0																	20.49
C.albicans_AI1RWKD		18.8																19.08
C.freundii_AIHSP9E			12.6															19.66
E.aerogenes_APEPRZ2				19.5														19.49
E.aerogenes_APFVKKY				20.1														19.98
E.aerogenes_APGZE6W				19.8														19.76
E.cloacae_APH49RU					19.8													20.10
E.coli_AIHSPV8						19.8												19.79
E.faecalis_AIGJRP0							18.9											19.04
E.faecium_APNKRGK								19.5										19.35
E.faecium_APPRJ2H								19.5										19.81
E.faecium_APRWEMF								20.0										20.08
K.oxytoca_AIBJZPH									12.8									19.37
K.oxytoca_AICSXVP									13.9									20.27
K.oxytoca_AIT97ZM									14.5									20.08
K.pneumoniae_AIAA1I9										19.2								19.33
K.pneumoniae_AIX0150										20.7								20.86
M.morganii_AIGJSED											20.4							19.15
M.morganii_AIHSQKL											19.9							19.06
M.morganii_AII1OFM											19.5							19.42
M.morganii_AII1OQT											20.9							19.59
M.morganii_AIKAMW1											30.6							19.69
M.morganii_APXGPXG											20.3							20.62
M.morganii_APYMJHE											19.4							19.76
P.aeruginosa_AIRSBQC												20.6						19.58
P.mirabilis_AIPAEY1											30.6		13.5					19.93
P.mirabilis_AIVI6G1								29.8		29.9			12.6					19.26
P.mirabilis_AIWR4M9													13.0					19.60
P.mirabilis_AP2W7M9													14.9					20.63
P.mirabilis_AP32Z76		30.5									30.5		13.3					19.96
P.mirabilis_AP47VT3													12.9					19.93
P.mirabilis_APZTD3C													12.2					19.00
P.stuartii_AIKAMLU														19.4				19.64
P.vulgaris_AIN1G7L				29.5										30.8	18.8			19.15
S.agalactiae_AIPAEK3		30.5														20.3		19.90
S.saprophyticus_AIQJC49																	20.7	20.39
Xeno_Ac00010014_a1												30.4		29.6				18.46
RNASEP_AIGJRHZ																		19.58

The UTM assays demonstrate high sensitivity and specificity with gDNA controls

Assays Demonstrate 5 Logs of Dynamic Range and Strong Linearity



Serial Dilution of Pooled ATCC gDNA Inclusivity Panel



UTM assays demonstrate 100 copies/ul sensitivity of sample input with gDNAs

Detection Objectives

- **Primary Objective**
  – Compare the ability of Guidance and traditional urine culture in detecting organisms causing a UTI
- **Secondary Objective**
  – Identify the frequency of observed polymicrobial infections and compare the ability of Guidance and traditional urine culture in detecting polymicrobial infections

Comparing Detection Levels

Guidance	Generation 3
Number of Patient Samples	196
Number Bacterial Organisms in Panel	25
Inclusion Criteria	DX Code for UTI from Urology Office
Exclusion Criteria	DX Code Not UTI

Comparison Study

Total Number of Cases	196
Total Number of Cases - Male	96
Total Number of Cases - Female	100
Total Number of Negative Cases	42
Total Number of Positive Cases	154

At Case Level Agree

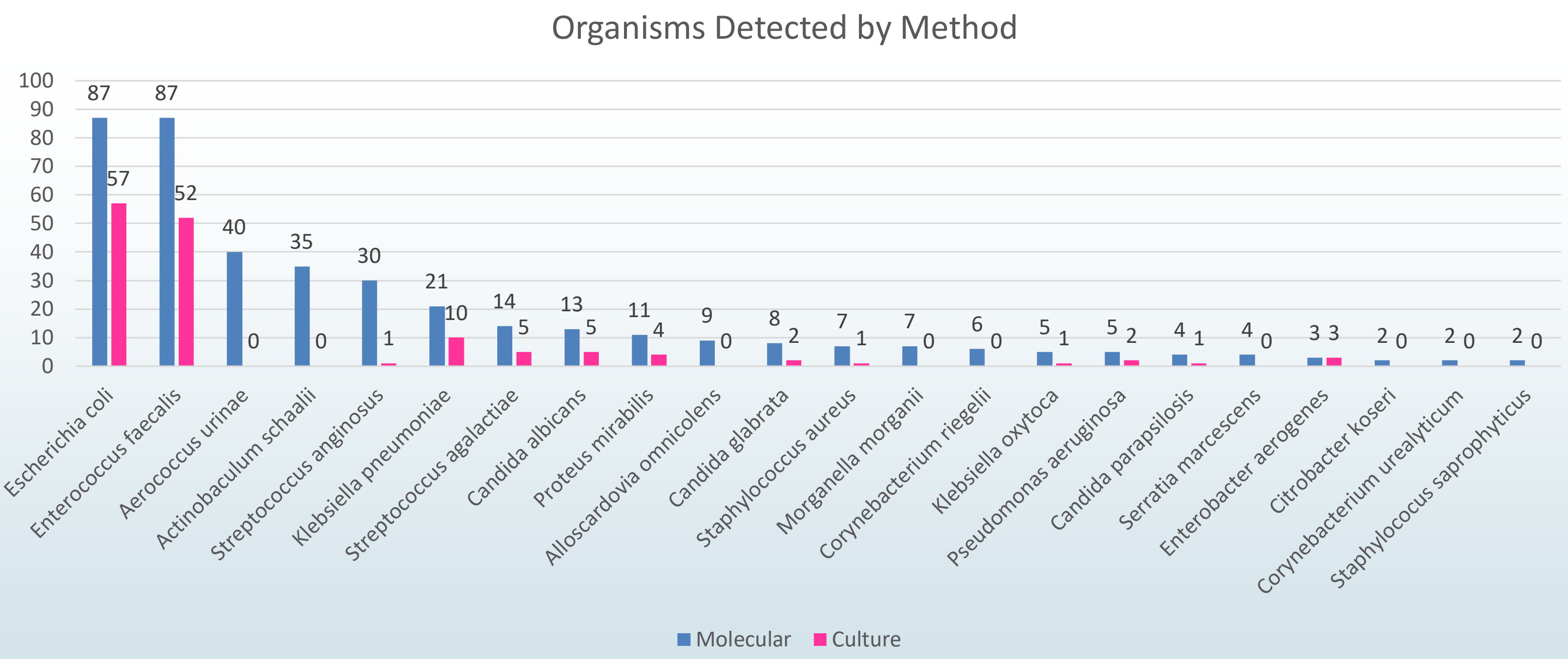
Missed by Guidance

		Guidance		
		Positive	Negative	Total
Culture	Positive	101	3	104
	Negative	50	42	92
	Total	151	45	196

Missed by Culture

Pathnostics
Moving Care Forward

Organisms Detected



Developing Methodologies for Managing Antibiotic Resistance Testing in a Polymicrobial Environment

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		

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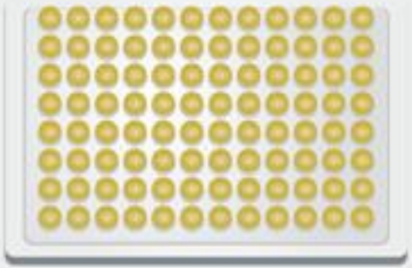
Phenotypic Testing

Media is plated into a 96-well plate, each with a different antibiotics

Urine sample (1mL minimum) added to each of the 96 wells and incubated

Urine in each well read by spectro-photometer for optical density (OD), which measures cell density

Determine potential antibiotic resistance or sensitivity based on OD (cell density)



Each well has different antibiotic

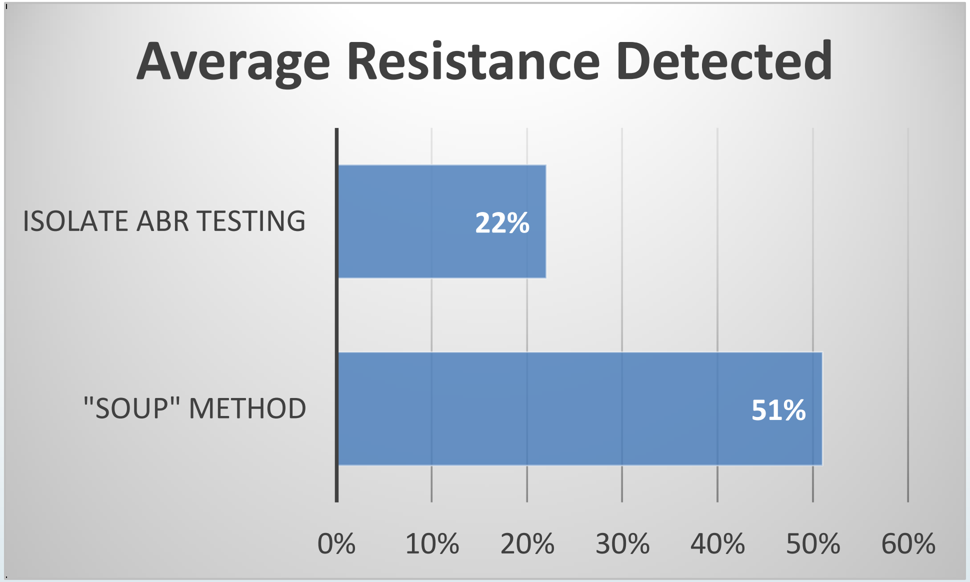
All of the bugs (polymicrobial)

Sample

Above threshold = Resistant

Below threshold = Susceptible

Antibiotic Resistance



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Moving Care Forward

THE GUIDANCE THERAPEUTIC MANAGEMENT SOLUTION

Identify symptomatic patients that require treatment

What is Causing the Symptoms?
Bacterial / Viral / Fungal

Identification

Therapeutic Solution

The Genetic Markers Are Found in the Urine That Indicate Resistance

Genotypic ABR Testing

Phenotypic ABR Testing

What Antibiotics Kill the Organisms in the Urine?

Conclusion

- UTI's Constitute 13B Impact to US Economy with Significant Morbidity and Mortality
- Urine is Not Sterile
- The Urinary Tract Contains a Microbiome That Plays an Important Role in Maintaining Health
- Polymicrobial Infections are Common and Result in Increased Rates of Virulence and Antibiotic Resistance
- Routine Urine Culture Has a High False Negative Rate and Miss the Majority of Uropathogens
- qPCR is a Powerful Tool for Identifying both the Identity of the Infectious Agent as well as the Presence of Antibiotic Resistance Genes
  - Presence of the ABR Genes Does Not Necessarily Correlate with Actual Resistence
- Phenotypic Assays Evaluating Pooled Resistance Allows for the Assessment of the Antibiotic Resistance of the Pooled Sample
- Combining Genotypic and Phenotypic Data Provides a Functional Answer with Respect to Both Fast and Slow Growing Organisms.

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