Pathnostics
Moving Care Forward

Guidance UTI Test

A Molecular Test For UTI Including Patented Proprietary Anti-Biotic Resistance / Sensitivity Technology

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Introduction: Guidance Test

Guidance Is Superior To
Standard Culture Test

Inpact of Polymicrobial Infections
On UTI & Antibiotic Resistance

Genotype Resistance Testing &
Antibiotic Sensitivity

Factors When Considering
Molecular Testing

Sample Patient Report

Pathnostics &
Leadership Team

Study: UTI & Elderly
UTI & Pregnancy

Guidance Molecular Test For UTI

In 2015, Pathnostics developed a proprietary molecular based test for UTI. It's name - Guidance UTI.

Compared to the traditional culture test, with Guidance UTI :

* The Detection Rate of Organisms Is Much Higher.
* The False Negative Rate Much Lower.
* More Polymicrobial Organisms Per Case Are Detected.
* Diagnostic Accuracy Is Higher.
* Utilizes Proprietary, Patented Technology For Anti-Biotic Resistance (ABR) Sensitivity Testing.
* Patient Reports List Detected Organisms and Anti-Biotics Based on Sensitivity and Clinical Data.
* Promotes Efficient and Accurate Anti-Biotic Stewardship.
* TAT Full Test Results Under 48 hours

By 1st Quarter 2019,
Full Results Will Be Available Within 12 Hours

GUIDANCE POINTS THE WAY TO QUICK AND EFFECTIVE RESOLUTION OF UTI INFECTIONS

Today's standard of care simply isn't good enough. Typical urine culture misses up to 2/3 of all UTI- positive patients and it detects organisms in only 4% of the cases to diagnose prostatitis.[1,2]

Now there's a better way to test and treat urologic infections. Guidance is a unique rapid molecular test for both pathogen identification and antibiotic sensitivity, backed by patented technology.

With quick 24-48 hour turnaround time, Guidance provides personalized therapy options that work the first time. So you can conquer your patients' unresolved infections.

KNOW WHAT'S CAUSING THE PROBLEM

Organism identification

- Tests for more pathogens
- Higher sensitivity and accuracy
- **Results in more accurate diagnosis**

KNOW HOW TO SUCCESSFULLY TREAT

Genotype resistance
+
Antibiotic sensitivity

- Tests more antibiotics
- Leverages dual assessments
- **Uncovers more effective, personalized therapy options**

The difference is clear

	GUIDANCE	OTHERS
Pathogens Tested	42	≤16
Antibiotic Resistance Genes	38	≤27
Pooled Phenotypic Sensitivity	YES	NO

1. Price TK, Dune T, Hilt EE, Thomas-White KJ, Kliethermes S, Brincat C, Brubaker L, Wolfe AJ, Mueller ER, Schreckenberger PC. The Clinical Urine Culture: Enhanced Techniques Improve Detection of Clinically Relevant Microorganisms J Clin Microbiol. 2016;54(5):1216-22.
2. Zegarra Montes LR, Sanchez Mejia AA, Loza Munarriz CA, Celis Gutierrez E. Semen and urine culture in the diagnosis of chronic bacterial prostatitis. Int Braz J Urol. 2008;34(1):30-40.

CHALLENGE 1

MISSED DIAGNOSIS

Standard urine culture misses up to 2/3 of all UTI-positive patients.[1]

GUIDANCE demonstrates a 26% increase in sensitivity.[3]

	GUIDANCE	URINE CULTURE
Positive Cases	200	148
Percentage Sensitivity	97%	71%

GUIDANCE improves diagnostic accuracy by more than 65%.[3]

	GUIDANCE	URINE CULTURE
Number Misdiagnosed	7	142
Percentage Misdiagnosed	4%	69%

CHALLENGE 2

POLYMICROBIAL INFECTIONS

In populations 65 and older, the mortality rate for those patients with symptomatic UTIs is as high as **33%**.[4] And the rate of polymicrobial UTIs is as high as **39%**.[5]

GUIDANCE detects 61% more organisms than culture by determining antibiotic sensitivity through phenotype testing (actual exposure of patient specimen to antibiotic).[3]

	GUIDANCE	URINE CULTURE
Number Detected	423	159
Percentage Detected	97%	36%

CHALLENGE 3

SLOW-GROWING PATHOGENS

Urine culture is less likely to detect pathogens that are difficult or slow to grow in culture, making treatment unnecessarily challenging.

GUIDANCE identifies antibiotic resistance genes from DNA through genotype testing (identification of pathogen DNA within pool of DNA isolated from detected organisms).

3. Data based on Pathnostics Laboratory internal studies comparing 300 cases of traditional urine culture vs. Guidance.
4. Cove-Smith A, Almond MK. Management of urinary tract infections in the elderly. Trends in Urology, Gynaecology & Sexual Health. 2007;12(4):31-4.
5. Laudisio A, Marinosci F, Fontana D, Gemma A, Zizzo A, Coppola A, Rodano L, Incalzi RA. The burden of comorbidity is associated with symptomatic polymicrobial urinary tract infection among institutionalized elderly. Aging Clin Exp Res. 2015;27(6):805-12.

WHO NEEDS GUIDANCE

Patients with:

- Recurrent UTI
- Interstitial cystitis
- Pyelonephritis
- Prostatitis

At-risk groups:

- Pregnant
- Elderly
- Past urinary culture results were "contaminated"
- On chronic pain care regimens
- Immunosuppressed
- Diabetic
- Men with UTI

TESTING OPTIONS

GUIDANCE BASIC

Tests for:

- Simple cystitis

Includes detection of:

- Bacterial and yeast organisms
- Bacterial groups
- Antibiotic resistant genes detected

GUIDANCE COMPREHENSIVE

Tests for:

- Recurrent, persistent, or complicated UTI
- Interstitial cystitis
- Prostatitis

Includes detection of:

- Bacterial and yeast organisms
- Bacterial groups
- Viral particles
- Antibiotic resistant genes detected

THE IMPACT OF POLYMICROBIAL INFECTIONS ON URINARY TRACT INFECTIONS & ANTIBIOTIC RESISTANCE

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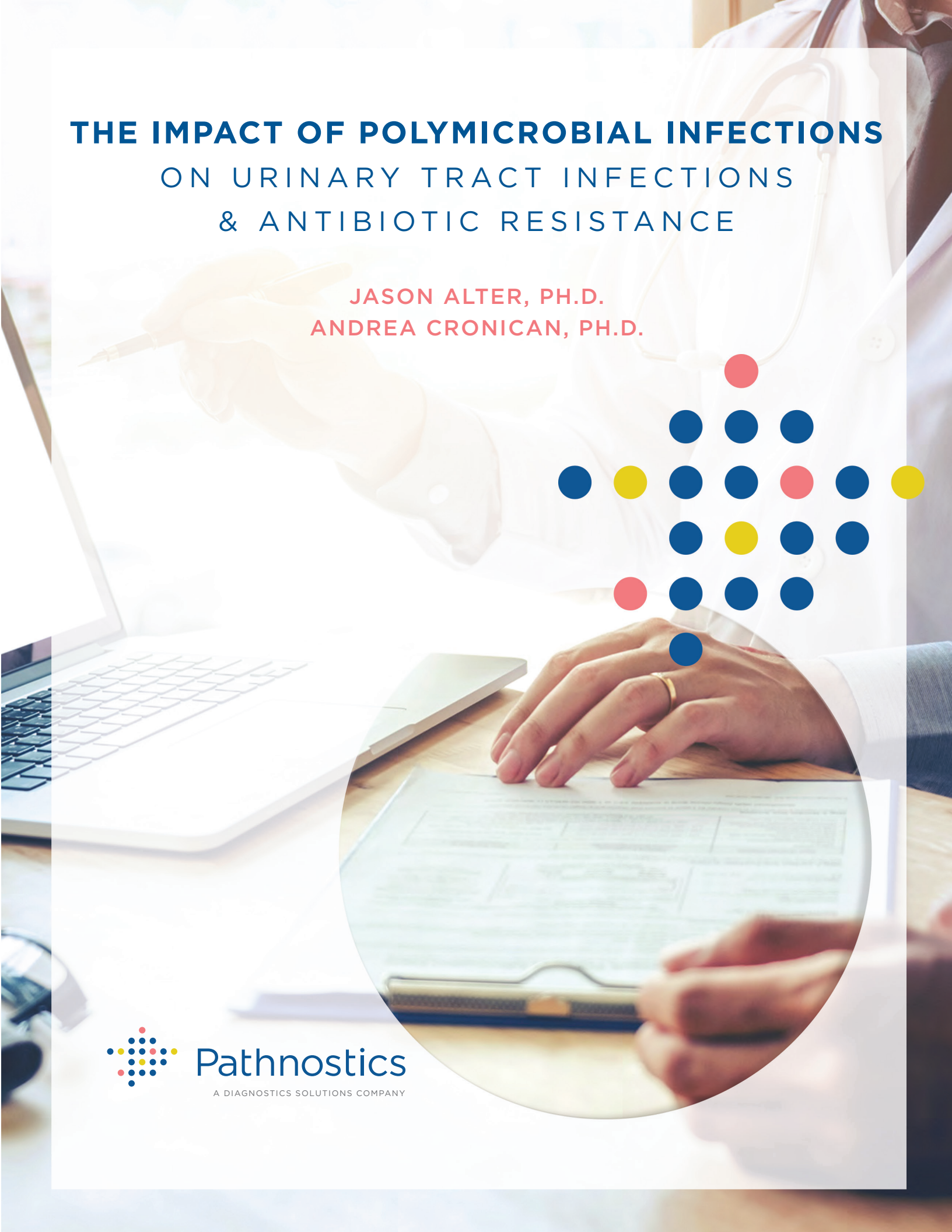

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A DIAGNOSTICS SOLUTIONS COMPANY

SYNOPSIS

- Traditional culture misses a significant percentage of organisms in urine which is no longeconsidered a 'sterile' environment.
- New information suggests that many infections are polymicrobial in nature and these mutualistic interactions can potentially enhance infection as well as resistance to antibiotics.
- GUIDANCE combines the latest technology in microbial DNA detection with a phenotypic, functional assay that provides clinicians with state of the art microbial assessment for UTIs.

TRADITIONAL CULTURE LIMITATIONS

Urinary tract infections (UTIs) result in ~10.5 million office visits in the US every year with an additional 1 million ER visits which result in approximately 100,000 hospitalizations.[1] Populations at risk include pregnant women, the elderly, patients with underlying urologic conditions, infants, etc. In elderly populations, genitourinary infections are the second most common infection with direct costs (in 1995) of $659 million and indirect costs of ~$936 million with total annual costs of $2 billion.[2,3] The introduction of molecular technologies, for the detection and characterization of microbes in the urinary tract is the necessary next step in the evolution of better clinical management of urinary tract disease.

The current gold standard for the detection of microbes contributing to UTIs is traditional culture which hasn't notably changed since it originated as meat infusion broths in 1865.[4] Robert Koch evaluated a number of solid media and settled on meat extract combined with gelatin poured on glass plates.[5] Subsequent research resulted in the incorporation of agar (polysaccharide derived from seaweed) to provide the solid support. Combined with the introduction of updated nutrients and modified glass supports, traditional agar plate media was in use by the 1890s. Although selective media has evolved over time, the general methodology has not significantly changed in a hundred years.

Traditional culture's reputation as the gold standard began to be challenged when molecular methods polymerase chain reaction (PCR) demonstrated that a wide variety of microbes, not found by culture, exist in the urine from female patients that did not present with the clinical definition of a UTI.[6] Urine, previously thought to be sterile, was now understood to have undetected microbes.[7] In hindsight, this should not have been a complete surprise because culture, by its nature, is biased towards faster-growing microorganisms (such as *Escherichia coli* or *Enterococcus faecalis*) that thrive in aerobic conditions, as opposed to slow growing organisms that are either fastidious or grow under anaerobic conditions (*Lactobacillus, Ureaplasma, etc.*).[8,9]

> "Culture misses 67% of uropathogens overall and even missed 50% of organisms in patients with severe urinary symptoms."

Enhanced culture methods were subsequently developed with the aim of detecting more and varied organisms; culture modification's included plating larger volumes of urine, varying atmospheric conditions and longer incubation periods. The enhanced culture approach (EQUC) found thirty-five genera and eight-five species, 92% of which were reported as no growth by the standard urine protocol.[10] In addition, a study by Loyola and the University of Texas expanded the already superior culture approach (EQUC) by additional plating conditions (BAP, chocolate, colistin-nalidixic acid agars and a variety of atmospheric conditions, etc.). They examined the flora from bladders of woman that did/did not meet the clinical UTI definition and discovered that traditional, current culture misses 67% of uropathogens overall and even missed 50% of organisms in patients with severe urinary symptoms.[11]

THE MICROBIOME

Although urine was, until recently, considered a sterile environment, significant research began to establish that microbes exist in communities in a variety of human systems such as the gut. In 2008, the National Institute of Health (NIH) initiated the Human Microbiome Project (HMP). The HMP mission is to provide the resources necessary to allow the wide-ranging characterization of the microbial communities of the human body (nasal passages, oral cavity, skin, gastrointestinal tract, and urogenital tract) and analyze their role in human health and disease-however, the bladder was not initially included in the project perhaps because it was assumed to be sterile or that it was considered unethical to obtain bladder biopsies or suprapubic aspirates from healthy individals.[8,12,13,] The investigation of a microbiome in the bladder lagged behind other systems because of the limited ability to culture microorganisms leading to a misunderstanding about the nature, frequency and scope of the bladder microbial community and its relationship to UTIs. However, we now know that the urinary tract has its own unique microbiota with multiple studies categorizing the urinary bacterial community.[9,13]

Using molecular tools, we now also know that the composition of a bacterial community can have clinical relevance. As an example, research demonstrates a substantial increase in *Lactobacillus* in interstitial cystitis (painful bladder syndrome). In culture, *Lactobacillus* requires 48 hours incubation in 7% CO^2 to be detected which may be why interstitial cystitis samples are not typically associated with bacterial growth.[14] In urgency urinary incontinence (UUI), an increase in UUI clinical severity is correlated with a decrease in diversity of the microbiota.[15]

POLYMICROBIAL INTERACTIONS

Traditionally, microbiological research has focused on mono-microbial infections – one organism at a time. This has largely been due to the relative ease of experimental approaches focused on one microbe ostensibly responsble for one infection. In reality, most infections are polymicrobial in nature - some studies have shown that as many as 39% of urine cultures from elderly patients have polymicrobial infections.[16] Understanding that infections are polymicrobial in nature is not new. Since the days of Pasteur, it was hypothesized that some microbes were not easily cultivated but still contributed to infection.[17] Polymicrobial infections can be distinguished in three ways: (1) Dysbiosis: changes in the composition of individual species within the bacterial community. (2) Pathogenic colonization (a pathogenic organism colonizes a site already inhabited by commensal microbes. (3) An organism colonizes a site it does not normally inhabit.

Bacterial communities inhabiting human sytems, such as the bladder, can be thought of as cities: three dimensional structures in which the location and spatial organization of the orgaisms plays an important role in virulence.[18] Some studies have even shown that mixed infections in biofilms are more robust to antimicrobial agents than individual species encased in a biofilm.[19] Other studies have demonstrated that *E. coli* can invade bladder epithelial cells and create intracellular bacterial reservoirs that can last for substantial periods of time.[20] Two or more of the individual species within these three dimensional *in situ* structures can result in worse disease when compared to either microbe acting alone: this is considered synergy and polymicrobial infections are considered to be worse than mono-microbial infections with experimental evidence in urinary tract infections in model systems as well as clinical isolates.[17,21] Croxall et al., compared clinical UTI isolates from patients with either poly- or mono-microbial infections and found that *E. coli* from mixed cultures were ~1,000x more invasive than the reference strain.[22] In UTIs with a primary uropathogenic microbe, other microbes at low levels ($<10^5$ CFU/mL of urine) have not been thought to be clinically relevant. However, Kline et al., have demonstrated that this a flawed thought process. In their murine-model research, low titers of group B *Streptococcus* may supress host immune processes creating a more favorable environment for uropathogenic organisms.[23]

Indeed, bacteria can not only interact with other bacterial species they can also cooperate with fungi to produce enhanced infections: *Candida albicans*, a fungal pathogen has been found to

coexist in polymicrobial infections in biofilm structures: these mixed infections are associated with amplified frequency and disease severity.[24] In the urinary tract, *E. coli* contributes to the ability of *C. albicans* to attach to the bladder lining which the organism was unable to do on its own. *Acinetobacter baumannii*, a hospital associated pathogen, also often interacts with *C. albicans* and is a problem for critically-ill patients on ventilation with urinary catheters.[25]

There are multiple ways discrete species act in synergistic ways to produce enhanced infections. Although, many organisms have distinct and separate metabolic pathways, experimental evidence in urinary infections that co-infection with *E. coli* and *Proteus mirabilis* enhances urinary tract colonization and pathogen persistence.[26] Additionally, species can cross feed: use metabolites from a different species, such as complex carbohydrates, as a fuel source.[27] Microbes also, produce low molecular weight signals (known as quorum sensing) that enable them to 'cross talk' with other cells of the same bacterial species to coordinate their activities. However, these signals can actually be listened to (eavesdropping) and answered by unrelated species.[17]

POLYMICROBIAL INFECTIONS IMPACT ON ANTIBIOTIC RESISTANCE

Antibiotic resistance is a well-known problem and currently contributes to ~23,000 deaths per year. Polymicrobial infections not only can enhance virulence, they also can effect changes in how infections respond to antibiotic therapy.[28] Bacteria become transcriptionally active during antibiotic treatment and research shows that antibiotics can impact the composition of the microbiome.[29] In UTIs, polymicrobial infections can often be considered probable contamination and not properly assessed. Combined with traditional culture's inability to detect many organisms, an incomplete picture of the patient's urinary microbial landscape can lead to ineffective therapy.

There are multiple mechanisms by which polymicrobial infections respond to antibiotic treatment– many of which remain to be elucidated. In some studies, three dimensional structures such as biofilms encapsulate bacteria in an extracellular matrix that can potentially provide protection against antibiotics.[24,30] *Staphylococcus aureus* encased in biofilms with *C. albicans* has been shown to have enhanced resistance to vancomycin.[31]

“Combined with traditional culture's inability to detect many organisms, an incomplete picture of the patient's urinary microbial landscape can lead to ineffective therapy.

Bacterial communication also plays an important role in mixed communities and can impact response to antibiotic therapy. In otitis media, signaling between bacterial species promotes resistance to antibiotics and persistence.[32] The intestinal pathogen, *Salmonella typhimurium*, becomes more antibiotic resistant in response to the signaling molecule indole produced by other bacterial species. Although *S. typhimurium* does not produce indole, when exposed to exogenous indole or indole produced by *E. coli* in a mixed community, *S. typhimurium* becomes more able to tolerate antibiotics. Indole signaling as well as other signaling mechanisms may affect the ability of pathogens in polymicrobial infections to resist antibiotics, persist and form chronic infections.[33] In mixed cultures, *Stenotrophomonas maltophilia* produces a diffusible signal factor that changes the morphology of neighboring *Pseudomonas aeruginosa* which can be associated to a specific gene (rpfF): this signaling factor produced by *S. maltophilia* increases the tolerance of *P. aeruginosa* to polymyxins B and E.[34] In model systems using 3D printing technology,

microscopic (picoliter) containers made of bovine serum albumin can be created that place poly-microbial species in defined proximity: the resulting interactions between *S. aureus* and *P. aeruginosa* have been shown to enhance the survival of *S. aureus* to β-lactam antibiotics.[35]

> “Clinical isolates often protected each other from the antibiotics.”

In urinary tract infections, polymicrobial in elderly patients typically consists of up to five organisms. De Vos et al., examined the interactions between 72 bacterial isolates from elderly people with UTI symptoms. They measured bacterial growth via optical density in an artificial urine medium and quantified the interactions between the species. They found that most interactions resulted in no change in growth but 18% of all species to species interactions enhanced growth and 40% of these positive interactions led to >2x growth. Conversely, 23% of interactions resulted innegative growth. They also measured the impact of species to species interactions on antibiotic efficacy. They assessed organism’s growth in response to two commonly used antibiotics for UTIs (trimethoprim-sulfamethoxazole and nitrofurantoin). Using media conditioned by donor isolates, they observed that clinical isolates often protected each other from the antibiotics: 25% of tested species to species interactions demonstrated greater than a 3.5 fold increase in tolerance for trimethoprim-sulfamethoxazole but decreases of the same magnitude only occurred in 12% of results. Similar results were observed for nitrofurantoin. They also noted that clinical isolates from the same community tended to protect each other slightly more than isolates from different communities.[36]

> “Species act in synergistic ways to produce enhanced infections.”

Table 1. GUIDANCE Pathogen Detection for UTIs

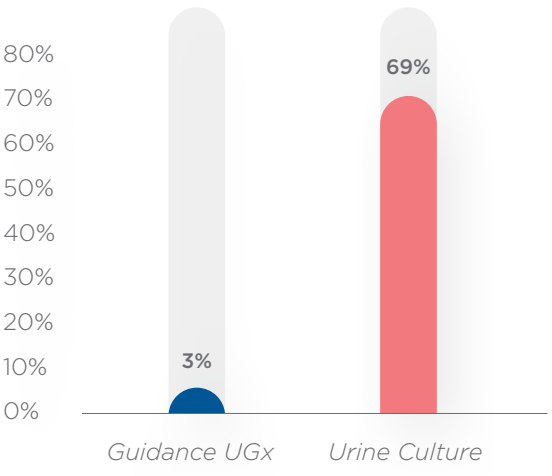
- *Acinetobacter baumannii*
- *Actinobaculum schaalii*
- *Aerococcus urinae*
- *Alloscardovia omnicolens*
- *Candida albicans*
- *Candida glabrata*
- *Candida parapsilosis*
- *Citrobacter freundii*
- *Citrobacter koseri*
- *Corynebacterium riegelii*
- *Corynebacterium urealyticum*
- *Enterobacter aerogenes*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Klebsiella oxytoca*
- *Streptococcus anginosus*
- *Morganella morganii*
- *Mycoplasma genitalium*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Serratia marcescens*
- *Staphylococcus aureus*
- *Staphylococcus saprophyticus*
- *Streptococcus agalactiae*

GUIDANCE MOLECULAR TESTING

GUIDANCE combines two critically important assessments to provide a nuanced view of the polymicrobial nature of UTIs and their potential response to a variety of possible antibiotic therapies. GUIDANCE uses polymerase chain reaction (PCR) to detect DNA from twenty-five (seven) pathogens for UTIs and twenty-seven pathogens for prostatitis (Table 1) and can detect organisms as low as 1,620 – 5,401 cells per milliliter (depending on the organism) to as high as >6 million cells/mL.

In an internal analysis, GUIDANCE was compared head-to-head to traditional urine culture (gold standard) for the ability to detect organisms causing UTIs. (All organisms detected by either method were at a threshold of 10,000 CFU of cells/ml). As seen in **Figure 1**, the GUIDANCE PCR-based assay had significant improvements in accuracy over culture in correctly finding pathogens in urine samples from UTIs: sensitivity (97% vs 31%), over culture with only 3% misdiagnosed compared to 69% for urine culture.

Misdiagnosed Positive Cases (N=207)



Accuracy (Positive Cases, N=207)

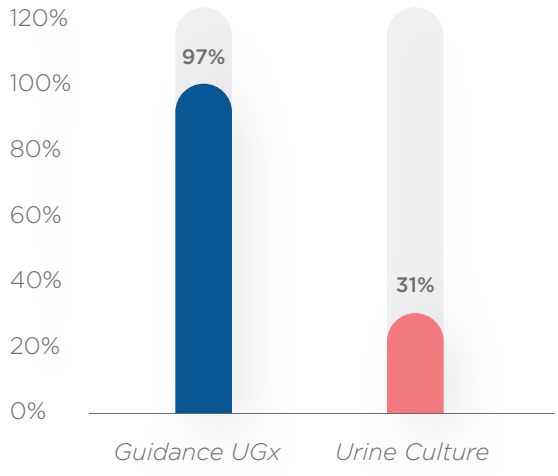


Figure 1. Although PCR is an important assessment of the presence of bacteria that cannot be detected by culture it is unable to assess if the DNA is from live versus dead cells (Rogers, 2010). Complementing GUIDANCE's PCR technology is a proprietary antibiotic resistance (ABR) assay which allows polymicrobial growth to be examined for functional antibiotic sensitivity.

Table 2.

- *Ampicillin*
- *Ampicillin/Sulbactam*
- *Augmentin*
- *Cefazolin*
- *Cefepime*
- *Cefoxitin*
- *Ceftazidime*
- *Ceftriaxone*
- *Cefaclor*
- *Ciprofloxacin*
- *Gentamicin*
- *Levofloxacin*
- *Nitrofurantoin*
- *Piperacillin/Tazobactam*
- *Tetracycline*
- *Trimethoprim/Sulfamethoxazole*
- *Vancomycin*

ANTIBIOTIC RESISTANCE (ABR) ASSAY

This assay measures optical density with a spectrophotometer setting a threshold value to measure growth of organisms in the 'soup', or polymicrobial mixture from the actual patient. The benefit of the 'soup' approach is that it allows real-world antibiotic sensitivity assessment of the polymicrobial community from the patient's UTI. Interaction between species that may result in unexpected antibiotic resistance may more likely be detected via this phenotypic assessment. The minimal inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in an agar or broth dilution susceptibility test. Optical density measurements greater than or equal to the threshold are designated Resistant (R) meaning bacterial organisms present in a patient sample were resistant to that particular antibiotic at that concentration. Measurements less than this threshold are designated Sensitive (S) meaning bacterial organisms present in a patient sample were sensitive to that particular antibiotic at that concentration. The GUIDANCE assay currently tests for eighteen antibiotics **(Table 2)** and the list continues to grow.

POLYMICROBIAL INFECTIONS ASSESSED WITH MOLECULAR TOOLS

As mentioned previously, some studies have shown that 39% of urine cultures from elderly patients have polymicrobial infections.[16] But our analysis of over two thousand cases suggests that polymicrobial infections occur much more frequently (Figure 2) with as many of 68% of cases having infections with more than one microbe. Indeed, 27% of these cases had at least four organisms. This is interesting in the context of the published data from De Vos et al, in which 18% of species to species interactions enhanced growth with 40% of these positive interactions leading to at least twice the growth as well as the fact that 25% of tested species to species interactions demonstrated greater than a 3.5-fold increase in tolerance for trimethoprim-sulfamethoxazole.[36]

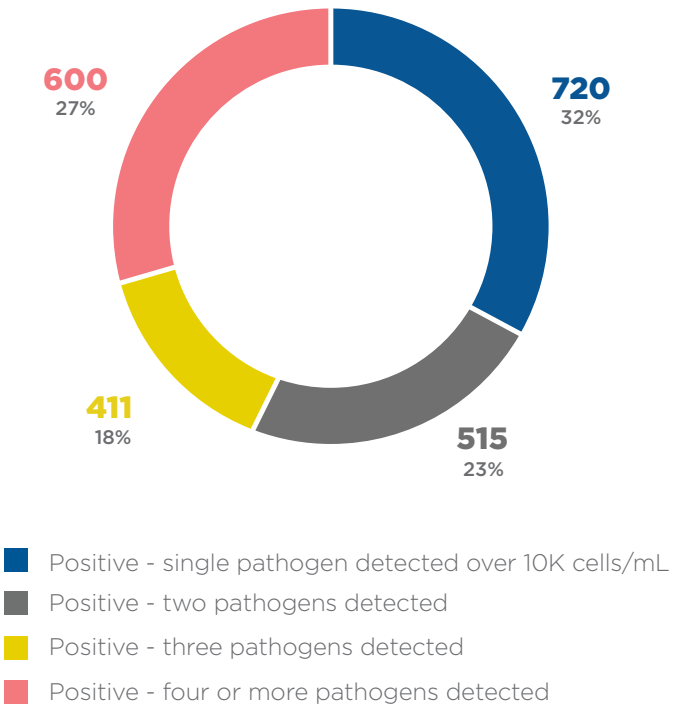


Figure 2. Summary of GUIDANCE urine testing for prostatitis (PRx) and urinary tract infection (GUIDANCE UGx) results. A total of 2,246 cases with infection were evaluated. Of these, 32% (720 of 2,246) were positive for a single pathogen while 23%, 18% and 27% of the cases were positive for 2, 3, or 4 or more pathogens.

% Occurrence of Co-Infections

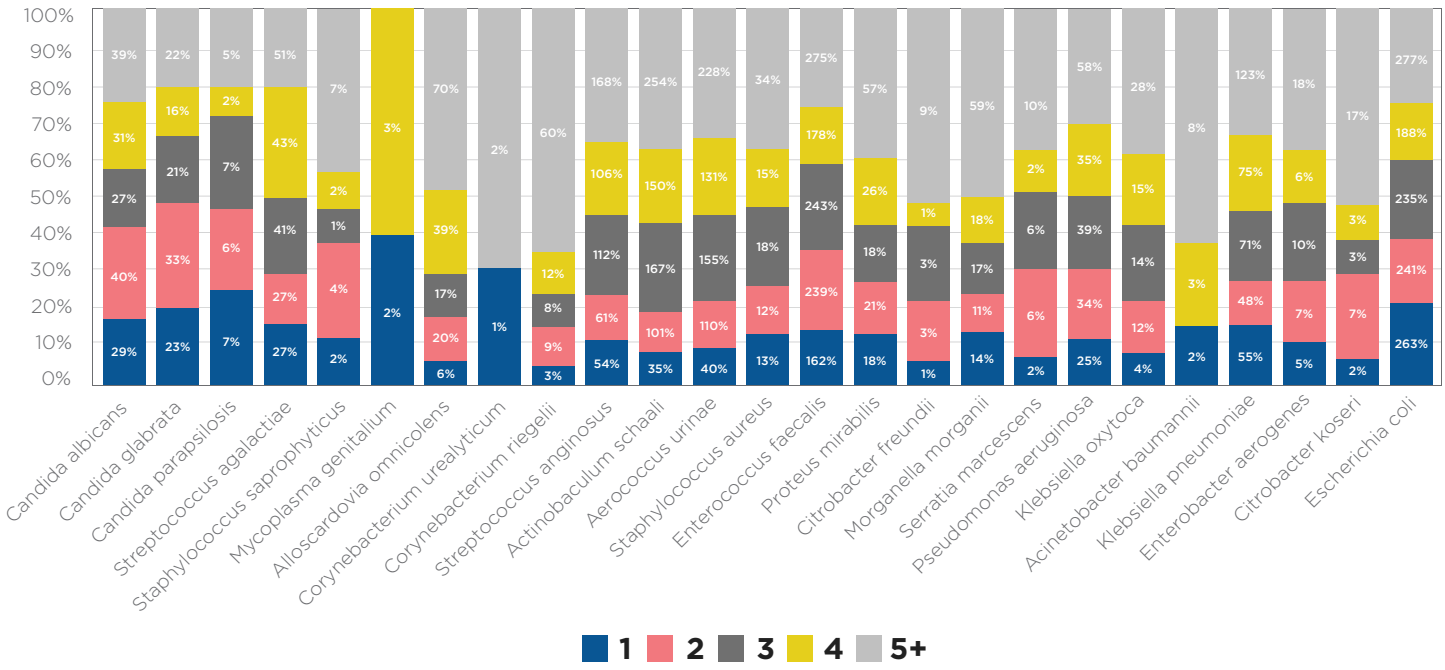


Figure 3. A review of co-infections using internal data shows interesting results.

A closer examination of laboratory co-infections (internal data) demonstrates that there is wide variability in both the percentage and composition of polymicrobial coinfections. *E. coli*, typically the most abundant organism in urinary tract infections had relatively equal distribution of coinfections from singular infections to as many as five organisms with the majority (78%) being polymicrobial. In some cases, exemplified by *Corynebacterium riegelli*, *Alloscardovia omnicolens*, *Proteus mirabilis*, Morganella morganii and *Klebsiella pneumoniae* - a significant percentage of infections had at least five organisms – 65%, 46%, 41%, 50% and 33% respectively. When reviewing fungal infections, the same results can be observed. As an example, 82% of *Candida albicans* infections were polymicrobial with 23% having five or more organisms (**Figure 3**).

In some cases (internal data), there is preliminary evidence of paired organisms having enhanced resistance to specific antibiotics compared to antibiotic resistance observed with either organism alone (**Figure 4a** and **4b**).

In **Figure 4a**, a mixture of *E. coli* and *Klebsiella pneumoniae* from patients with symptomatic urinary tract infection were associated with enhanced resistance to Ampicillin/Sulbactam and Piperacillin/Tazobactam combinations. In **Figure 4b**, a mixture of *E. coli* and *Enterococcus faecalis* from patients with symptomatic urinary tract infection were associated with enhanced resistance to Ampicillin or Nitrofurantoin.

These results highlight how polymicrobial infections may negatively impact on clinical management and the importance of using the proper assay. Mutualistic infections resulting in increased antibiotic resistance would not be captured by culture or simple DNA detection which assesses the presence or absence of resistance genes: only a molecular assay, like GUIDANCE which does both - combines DNA detection with phenotypic characterization - would provide the most complete information to the clinician.

Figure 4a. Internal data

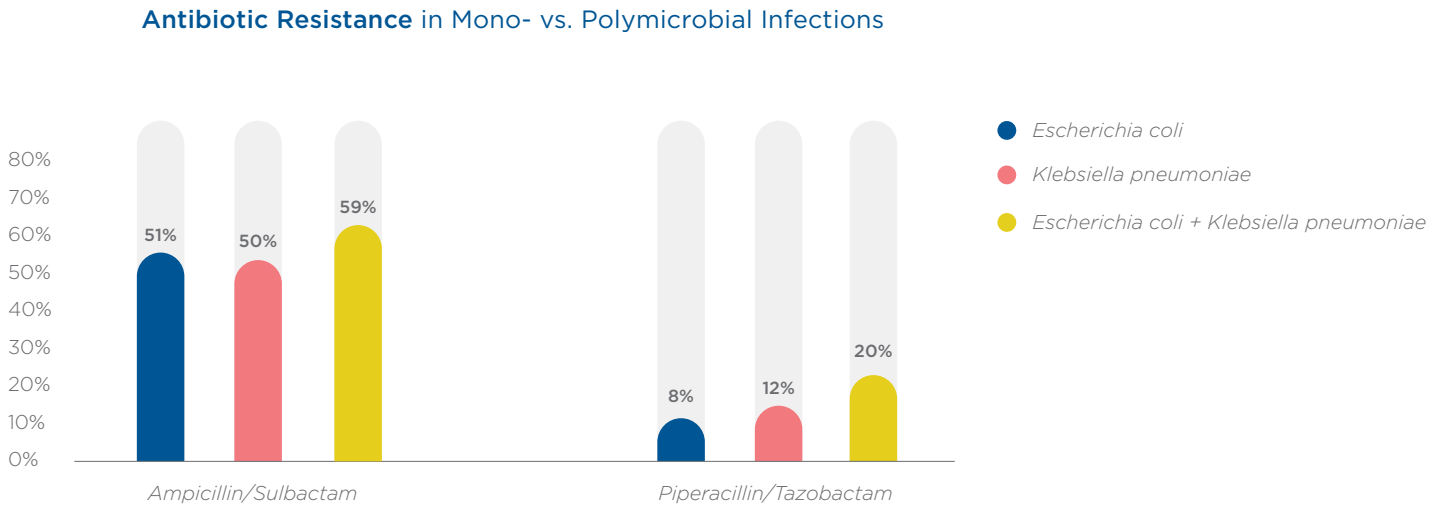
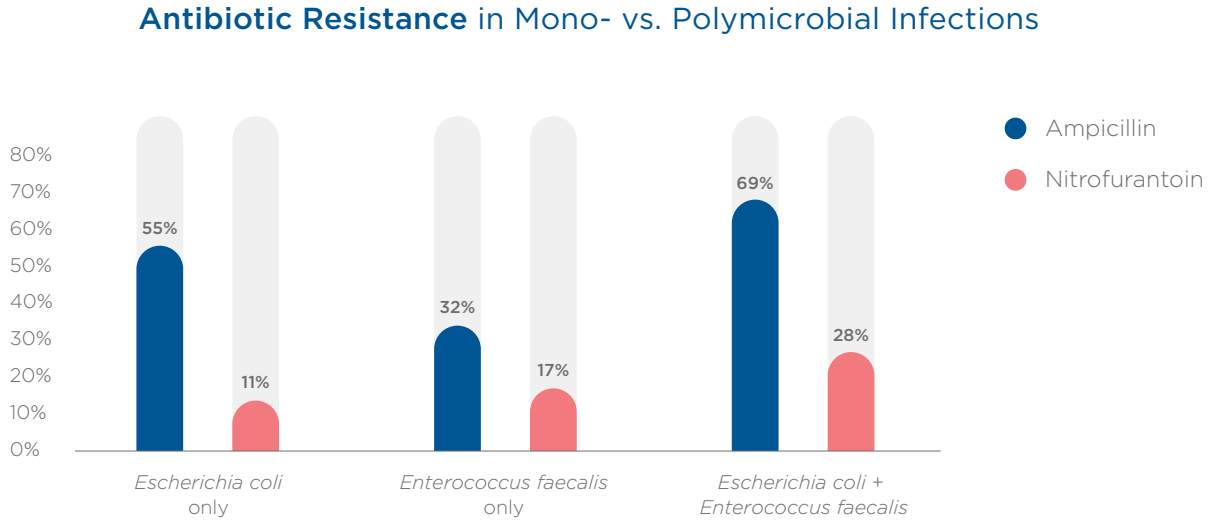


Figure 4b. Internal data



ANTIBIOTIC RESISTANCE (ABR) ASSAY

In the United States, UTIs result in millions of office and ER visits with annual costs in excess of $1 billion. Although culture, the current laboratory gold standard for microbe detection, hasn't significantly changed since the mid-nineteenth century, our scientific knowledge and perspective on urine as a sterile environment has evolved. Urine is no longer considered sterile and now is thought to have a microbiome in which infections are polymicrobial and mutualistic in nature: this has implications for antibiotic resistance. Current culture misses ~67% of uropathogens and new approaches are required. The GUIDANCE molecular assay has demonstrably higher pathogen detection than culture and combined with its unique phenotypic assay component, GUIDANCE allows for more informed patient management.

Pathnostics

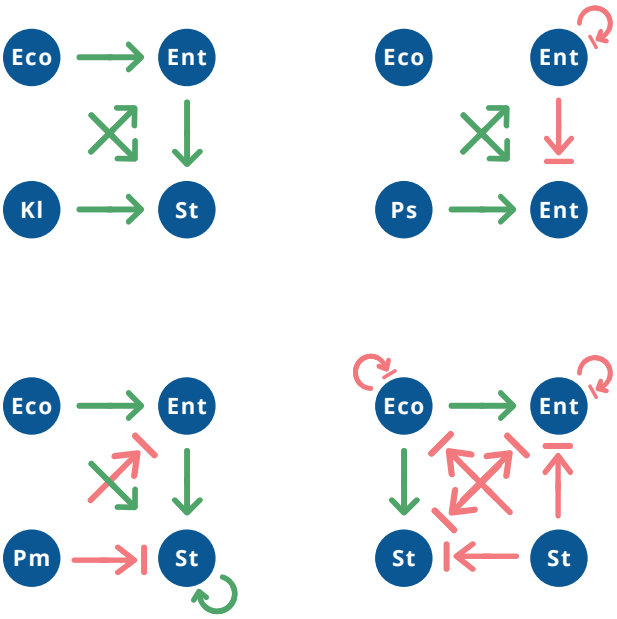
THE HIDDEN WORLD OF THE BLADDER MICROBIOME

UTI polymicrobial infections create an ecological environment in which the organisms found in the community impacts each other's;

- Proliferation rate
- Virulence potential
- Overall survival in the presence of antibiotics

STABLE COMMUNITY FORMATION THROUGH CROSS-FEEDING

Organisms produce metabolites that nourish the other members of the community.[1]



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)

IMPROVED SURVIVAL RATE WITH ANTIBIOTICS

Increased Survival In The Bladder Lumen

Within mouse models, bladders first infected with *Group B Streptococcus (GBS)* down regulates the host immune response through the release of capsule sialic acids allowing co-infected UPEC to survive within the lumen of the bladder as such alter the long-term outcomes of the infection as there is an increased risk of ascending kidney infections.[2]

Increased Risk Of Ascending Pyelonephritis

Ascending pyelonephritis was caused by *Staphylococcus saprophyticus* when co-infected with *Proteus mirabilis* both by inoculating the organisms separately and in combination into rat bladders. Microbial cultures of tissue homogenates revealed that pyelonephritis by both bacteria occurred significantly more often in rats precisely when the two organisms were instilled together, implying a synergistic virulence between the two species.[3]

Increased The Risk Of Urinary Stones

In mouse models, coinfection with *P. mirabilis and P. stuartii* led an increased occurrence of urinary stones.[4]

IMPROVED OVERALL SURVIVAL IN THE PRESENCE OF ANTIBIOTICS

Organism Interactions Affect Antibiotic Tolerance

Antibiotic tolerance is the ability of the bacterial population to survive transient exposure of antibiotics even at concentrations that far exceed MIC levels. As such, longer exposure rather than higher concentrations of antibiotics is required to produce the same level of killing in a tolerant strain. Tolerance occurs through the triggering of bacteria to grow more slowly. Tolerance can increase >3.5 fold based upon the organism interactions. For example, *P. mirabilis* has a protective effect on other organisms in the presence of antibiotics but mostly harms others in the absence of antibiotics.[1]

One Strain Protects The Entire Populations Of Bacteria

Resistance, alternatively, is used to describe the inherited ability of microorganisms to grow in the presence of high concentrations of an antibiotic, irrespective of the timeframe of treatment. In many cases, resistant cells inactivate the antibiotic, decreasing its extracellular concentration by breaking down the antibiotic, which subsequently, assists the whole bacterial population.[5]

Bacterial Organisms Share Resistance Genes Through Horizontal Gene Transfer (HGT)

HGT is the movement of genetic material between organisms, including different species. Research studies indicate that horizontal gene transfer serves a greater role than clonal expansion in the rise of antibiotic resistance levels.[6-7]

QUESTIONS?

Contact Joe Diange at Cell: 813-781-2520 or Email: joediange@gmail.com

References:

1. De Vos, M., Zagorski, M., McNally, A. and Bollenbach, T. 2017. Interaction networks, ecological stability, and collective antibiotic tolerance in polymicrobial infections. *Proceedings of the National Academy of Sciences*. 114, 40 (2017), 10666–10671.
2. Kline, K., Schwartz, D., Gilbert, N., Hultgren, S. and Lewis, A. 2012. Immune Modulation by Group B Streptococcus Influences Host Susceptibility to Urinary Tract Infection by Uropathogenic Escherichia coli. *Infection and Immunity*. 80, 12 (2012), 4186–4194.
3. Hjelm E, Lundell-Etherden I, Mårdh PA. Ascending urinary tract infections in rats induced by Staphylococcus saprophyticus and Proteus mirabilis. Acta Pathol Microbiol Immunol Scand [B]. 1987; 95:347–350.
4. Armbruster CE, Smith SN, Yep A, Mobley HL. Increased incidence of urolithiasis and bacteremia during Proteus mirabilis and Providencia stuartii coinfection due to synergistic induction of urease activity. J Infect Dis. 2014; 209:1524–1532
5. Yurtsev, E., Chao, H., Datta, M., Artemova, T. and Gore, J. 2013. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. *Molecular Systems Biology*. 9, 1 (2013), 683.
6. Blahna, M., Zalewski, C., Reuer, J., Kahlmeter, G., Foxman, B. and Marrs, C. 2006. The role of horizontal gene transfer in the spread of trimethoprim–sulfamethoxazole resistance among uropathogenic Escherichia coli in Europe and Canada. *Journal of Antimicrobial Chemotherapy*. 57, 4 (2006), 666–672.
6. Andrup, L and Microbiology, A.-K. 1999. A comparison of the kinetics of plasmid transfer in the conjugation systems encoded by the F plasmid from Escherichia coli and plasmid pCF10 from Enterococcus.... *Microbiology*. (1999).

GUIDANCE IS THE BEST OF BOTH WORLDS

Only Guidance combines both **genotype resistance testing** and **antibiotic sensitivity** on the collection of pathogens to identify the most effective therapy choices in a polymicrobial environment.

Three reasons why genotype resistance testing alone isn't enough:

1

Too many resistance genes to put in one assay. Scientists have identified tens of thousands of resistance genes, impacting hundreds of antibiotics in hundreds of organisms.[6] Unfortunately, there is a limited number of resistance genes that can be identified via molecular assay.

2

Resistance genes continuously evolve and are shared. Bacteria generate new resistance genes and then transfer those genes to other bacteria on a regular basis. So the ability to identify and characterize new resistance genes will always lag behind bacteria's capability to create them.[7]

3

Resistance gene may not be functional. Even after the gene is characterized, the actual presence of the resistance gene doesn't guarantee the gene is functional and exerting resistance within the detected pool of bacteria.[8]

6. Liu B, Pop M. ARDB—Antibiotic Resistance Genes Database. Nucleic Acids Res. 2009;37:D443-7
7. Choi SM, Kim SH, Kim HJ, Lee DG, Choi JH, Yoo JH, Kang JH, Shin WS, Kang MW. Multiplex PCR for the Detection of Genes Encoding Aminoglycoside Modifying Enzymes and Methicillin Resistance among Staphylococcus Species. J Korean Med Sci. 2003;18(5):631-6.
8. Martineau F, Picard FJ, Lansac N, Ménard C, Roy PH, Ouellette M, Bergeron MG. Correlation between the Resistance Genotype Determined by Multiplex PCR Assays and the Antibiotic Susceptibility Patterns of Staphylococcus aureus and Staphylococcus epidermidis. Antimicrob Agents Chemother. 2000;44(2):231-8.

Pathnostics
Moving Care Forward

Phenotype and Genotype

Phenotype Advantages	Genotype Advantages
Takes into account mutualism among organisms in polymicrobial infections.	**Can detect resistant genes for slow growing pathogens**
Pathogens present are exposed to antibiotic, thereby obtaining accurate resistance/sensitivity info based on functional/active pathogens.	Provides Direct Access to a Pool of DNA
ABR testing can provide resistance/sensitivity info even if there may be pathogens not identified	Easy to Understand the Results-the Target is Either There or Not There

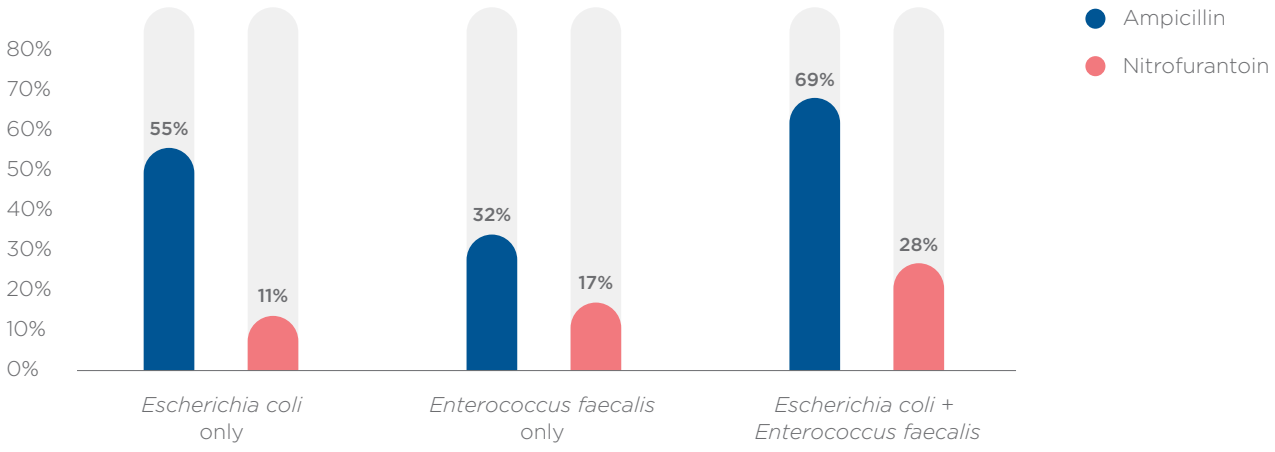
MUTUALISM 101

In elderly patients' UTIs, polymicrobial infections typically consist of up to five organisms. These infections create an ecological environment in which the **organisms enhance each other's proliferation rates and overall survival in the presence of antibiotics.**[9-13]

Bacterial communities thrive due to cross-feeding because organisms produce metabolites that nourish the other members of the community.[10]

More pathogens means more chances to evade treatment.
With a superior phenotype testing process for antibiotic resistance, Guidance results in improved treatment for polymicrobial infections.

Antibiotic Resistance in Mono- vs. Polymicrobial Infections[14]



9. De Vos MGJ, Zagorski M, McNally A, Bollenbach T. Interaction networks, ecological stability, and collective antibiotic tolerance in polymicrobial infections. Proc Natl Acad Sci U S A. 2017;114(40):10666-71.
10. Yurtsev EA, Chao HX, Datta MS, Artemova T, Gore J. Bacterial cheating drives the population dynamics of cooperative antibiotic resistance plasmids. Mol Syst Biol. 2013;9(1):683.
11. Blahna MT, Zalewski CA, Reuer J, Kahlmeter G, Foxman B, Marrs CF. The role of horizontal gene transfer in the spread of trimethoprim–sulfamethoxazole resistance among uropathogenic Escherichia coli in Europe and Canada. J Antimicrob Chemother. 2006;57(4):666-72.
12. Andrup L, Andersen K. A comparison of the kinetics of plasmid transfer in the conjugation systems encoded by the F plasmid from Escherichia coli and plasmid pCF10 from Enterococcus faecalis. Microbiol. 1999;145(8):2001-9.
13. Croxall G, Weston V, Joseph S, Manning G, Cheetham P, McNally A. Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. J Med Microbiol. 2011;60(1):102-9.
14. Data on file.

HERE'S HOW YOU ACCESS YOUR PERSONALIZED THERAPY OPTIONS

CLINICAL UTILITY

PATHOGEN IDENTIFICATION

Polymerase chain reaction (PCR) amplification-based assay to test up to 42 pathogens

THERAPEUTIC OPTIONS

- Targeted detection of 38 resistance genes spanning 7 different antibiotic classes
- Phenotypic data showcasing antibiotic sensitivity for polymicrobial environment
- Personalized antibiotic options based on supportive evidence

INTERPRETATION

DETECTED PATHOGENS

Cells of organisms per milliliter of sample:

- 10,000-49,999
- 50,000-99,999
- ≥100,000

Detection range as low as 500 cells/mL (depending on organism) to 6,000,000 cells/mL.

Viral and STD pathogens will be noted as "detected" or "not detected" only.

ANTIBIOTIC RESISTANCE

- **Sensitive:** antibiotic prevented growth of polymicrobial culture
- **Resistant:** antibiotic did not prevent growth in polymicrobial culture
- **RGI:** resistance gene identified

SPECIMEN

URINE

Voided urine or catheter urine collected within 5 days

Samples that were frozen, have PreservCyt, or were collected with Foly catheter tips will be rejected.

TEST PERFORMANCE

	SENSITIVITY	SPECIFICITY
PATHOGEN DETECTION	97%	100%
ANTIBIOTIC RESISTANCE	95%	89%

VALUE OF COMBINED GENOTYPE AND PHENOTYPE TESTING FOR GUIDING TREATMENT SELECTION

Genotype
Informs what genes are present

Phenotype
Shows which antibiotics effectively kill the organisms

The gene may be present ...

Resistance genes
Many are still undiscovered or not measured

... but it's what is "turned on" that matters.

Resistant organisms

Has resistance genes but are not resistant

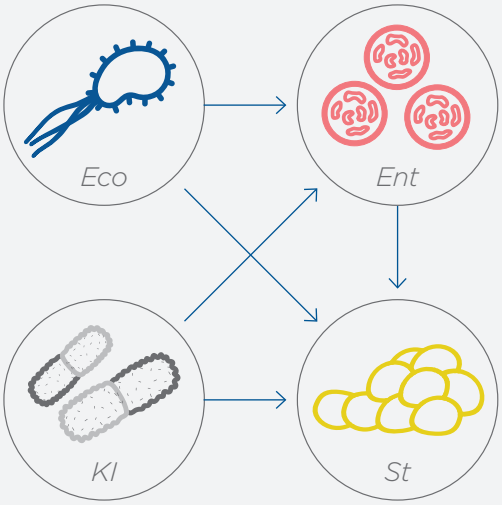
In real clinical data, even when testing as many as 38 resistance genes, genotypic and phenotypic results disagree nearly half of the time. To get all the data you need to choose the best treatment path, it's imperative to test both, not just one or the other. **Our Guidance tests do exactly that.**

PHENOTYPE VS GENOTYPE RESULTS

42% DISAGREE

KEEP MUTUALISM IN MIND

Pathogens thrive in polymicrobial infections because they benefit from cross-feeding and antibiotic resistance sharing. That makes it essential to test the **pooled antibiotic sensitivity** of pathogens in an infection to select treatment options with the highest chance of success.



GET GUIDANCE FOR YOUR PATIENTS

By combining targeted patient-specific data with known clinical literature and highlighting the best therapy options in easy-to-interpret reports, Pathostics helps you conquer your patients' diagnostic and treatment dilemmas.

PATHNOSTICS.COM | 714.966.1221

GUIDANCE TEST DETAILS

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

POOLED PHENOTYPE ANTIBIOTIC RESISTANCE/SENSITIVITY TESTING INCLUDES:

- Ampicillin (PO/IV)
- Ampicillin/Sulbactam (IV)
- Amoxicillin/Clavulanate (PO)
- Cefaclor (PO)
- Cefazolin (IV)
- Cefepime (IV)
- Cefoxitin (IV)
- Ceftazidime (IV)
- Ceftriaxone (IM/IV)
- Cefuroxime (PO)
- Cephalalexine (PO)
- Ciprofloxacin (PO/IV)
- Gentamicin (IM/IV)
- Levofloxacin (PO)
- Meropenem (IV)
- Nitrofurantoin (PO)
- Piperacillin/Tazobactam (IV)
- Tetracycline (PO)
- Trimethoprim/Sulfamethoxazole (PO/IV)
- Vancomycin (IV)
- Meropenem/Vaborbatum (IV)

POOLED GENOTYPE ANTIBIOTIC RESISTANCE GENES INCLUDE:

- Ampicillin
- ß-Lactamase
- Carbapenem
- Macrolide
- Methicillin
- Quinolinone/Fluoroquinolone
- Vancomycin

*Coagulase neg. staphylococci: *Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus lugdunenesis, Staphylococcus saprophyticus*

**Viridans group streptococci: *Streptococcus anginosus, Streptococcus oralis, Streptococcus pasteuranus*

Pathnostics

EFFECTIVE UTI MOLECULAR TESTING: 4 KEY FACTORS TO CONSIDER

Detection Capabilities

- How many pathogens can be detected?
- Are the results quantitative at absolute levels?
- How timely are the results?
- Does it provide unnecessarily redundant information?

Relevant Information

- Does it detect the common causative agents for UTI?
- Does it report nonpathogenic or rare bacteria with unclear treatment implications?

Treatment Guidance

- Are they using genotypic AND phenotypic data?
- Do they evaluate antibiotic sensitivity individually per pathogen or in a polymicrobial environment?
- Is the literature guidance limiting therapeutic options due to the population on which it's based?

Intuitive Report

- Can you easily interpret the best therapeutic path to take?
- How many report parameters are customized per patient result?

REVIEW YOUR TESTING OPTIONS

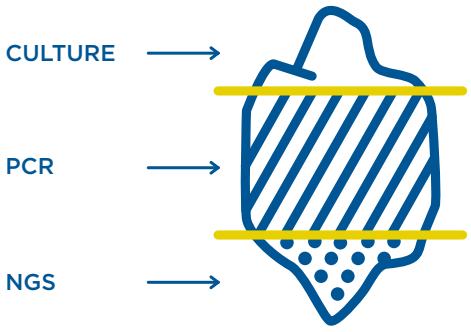
Today's leading urinary tract infection (UTI) molecular testing methods use a combination of polymerase chain reaction (PCR) with either next-generation sequencing (NGS) or pooled antibiotic sensitivity.

Here's what's important to get from your UTI molecular testing approach:

		PCR + NGS		PCR + POOLED SENSITIVITY
		PCR	NGS	
Detection Capabilities	Number of pathogens detected:	<10	25K organisms	42
	Quantify absolute levels:	YES	NO (% only)	YES
	Timely results:	~24 hours	72 - 120 hours	24 - 48 hours
Relevant Information	Microbiomes are everywhere, and not all organisms are detrimental or at a level of concern.	Detects all known microbes including normal (non-pathogenic) flora		Targets known pathogens associated with symptomatic UTI with absolute concentration level (as opposed to relative with NGS)
Treatment Guidance	Potential resistance (genotype):	YES		YES
	Number of genes tested:	15		38
	Tested sensitivity (phenotype):	NO		YES
	Number of antibiotics tested:	0		18
	Singular view vs pooled environment:	N/A		Pooled
	Literature treatment guidance based on appropriate population	NO		YES
Intuitive Report	Highlights best treatment options to address ALL pathogens present	NO		YES
	Number of dynamically customized data types within report	2 (detects pathogens + resistance genes)		3 (detects pathogens + resistance genes + pooled sensitivity)

How deep do you really need to go?

Standard culture only addresses the tip of the iceberg for UTI diagnosis. While PCR captures the majority of known causative agents, NGS detects all known microorganisms whether they're detrimental or not.



Common UTI causative agents requiring treatment

TECHNOLOGY COMPARISON

Because both NGS and PCR have pros and cons, it's important to consider the context of use and which would be best for that particular application.

NGS	VS	PCR
medium 	ACCURACY	 high
10^5	# OF PATHOGENS DETECTED	1 - 50
low 	SENSITIVITY	 high
$ $ $	COST	$
days - weeks 	TURNAROUND TIME	 hours - days
detect presence + sequence	A A T G C	DATA TYPE  detect presence + quantity
abundant 	AMOUNT OF DATA	 targeted
	INFORMATICS	

GUIDANCE COMPREHENSIVE RECURRENT, PERSISTENT, OR COMPLICATED UTI REPORT

Pathnostics

Patient: Mrs. Smith
DOB: 08/04/1935
Gender F
Phone: (941) 888-8888
MRN#:

Ordering Physician: M Welby, MD
Facility: Urology Colleagues
Phone: (941) 444-4444
Fax: (941) 777-7777

Case#: PUXR18-4444
Collection Method: Voided
Date Collected: 09-13-2018
Date Received: 09-14-2018
Date Reported: 09-16-2018

RESULTS: PATHOGENIC DNA DETECTED

Organism(s) Tested - Detected:

Enterococcus faecalis >100,000 cells/mL
Klebsiella pneumoniae >100,000 cells/mL
Morganella morganii >100,000 cells/mL
Pseudomonas aeruginosa <10,000 cells/mL

See page 3 for organism(s) tested - not detected

Antibiotic Sensitivity Detected:

Ampicillin / Sulbactam (IV)
Cefepime (IV)
Cefoxitin (IV)
Ceftazidime (IV)
Ceftriaxone (IM/IV)
Ciprofloxacin (PO/IV)
Gentamicin (IM/IV)
Levofloxacin (PO/IV)
Meropenem (IV)
Nitrofurantoin (PO)
Piperacillin / Tazobactam (IV)
Sulfamethoxazole / Trimethoprim (PO/IV)

Antibiotic Resistance Detected:

Amoxicillin / Clavulanate (PO)
Ampicillin (PO/IV)
Cefaclor (PO)
Cefazolin (IV)
Tetracycline (PO)
Vancomycin (IV)

Antibiotic Resistance Genes Detected:

Macrolide Resistance
ESBL Resistance
Ampicillin Resistance

GUIDANCE COMPREHENSIVE
RECURRENT, PERSISTENT, OR COMPLICATED UTI REPORT

Pathnostics

Patient: Mrs. Smith **DOB:** 08/04/1935 **Case#:** PUXR18-4444

	Amoxicillin / Clavulanate	Ampicillin	Ampicillin / Sulbactam	Cefaclor	Cefazolin	Cefepime	Cefoxitin	Ceftazidime	Ceftriaxone	Ciprofloxacin	Gentamicin	Levofloxacin	Meropenem	Nitrofurantoin	Piperacillin / Tazobactam	Sulfamethoxazole / Trimethoprim	Tetracycline	Vancomycin
Formulations	PO	PO/IV	IV	PO	IV	IV	IV	IV	IM/IV	PO/IV	IM/IV	PO/IV	IV	PO	IV	PO/IV	PO	IV
Pooled Phenotypic Sensitivity	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R
MIC Results (ug/mL)			8/4			1	4	4	1	1	4	1	1	32	16/4	2/38		
Pooled Resistance Gene(s) Identified	RGI	RGI	RGI	RGI	RGI	RGI	RGI	RGI	RGI						RGI	RGI		
Bacterial Organism(s)																		
Enterococcus faecalis	✓	✓	✓							✓	✓	✓	✓	✓	✓			✓
Klebsiella pneumoniae	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		
Morganella morganii	✓			✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		
Pseudomonas aeruginosa				✓		✓	✓	✓		✓	✓	✓	✓		✓			

✓ = Supportive Evidence S = Sensitive R = Resistant **RGI** = Resistance Gene Identified

Pathnostics Laboratory

Pathnostics is a diagnostic solutions company that pioneers approaches for better patient medical care. Founded in 2011, the 30,000 sf laboratory located in Irvine, California specializes in anatomic, clinical and molecular pathology. In only a few years, Pathnostics has evolved into a leading edge, full-service, reference and esoteric testing laboratory. Our team of experts develop solutions to address diagnostic and therapeutic dilemmas including an emphasis on antibiotic stewardship because we care about a better future for physicians, patients, and the health care system.

At Pathnostics we believe there is a growing need in the highly specialized areas of Molecular Pathology, Immunohistochemistry (IHC) and Fluorescence In-Situ Hybridization (FISH) testing. Currently our attention is focused on the early detection and treatment of Cervical Cancer, Endometrial Cancer, Uterine Cancer, Breast Cancer, Bladder Cancer, Prostate Cancer, Colon Cancer, and Esophageal Cancer. Our cutting-edge research, development of new assays, and refinement of existing diagnostic tests, produces efficiency and clinical value for reliable and cost-effective patient assessment and treatment.

Recently, Pathnostics developed a molecular test for chronic, recurrent, and complicated UTI's. It quickly diagnosis the cause of the (typically) polymicrobial UTI with a TAT of under 48 hours. Its proprietary, patent-pending antibiotic resistance sensitivity technology increases the odds for it to recommend the correct antibiotics and eradicate the UTI the first time. It is far superior to the standard culture test that physicians have depended on for over 100 years. We believe it is also superior to any other molecular test for UTI currently offered in the marketplace.

Pathnostics prides itself on personalized service, excellent quality and the utmost patient care. Everything we do is calculated including hours of business and lab operation, customer care, accessibility of clinical professionals, turn-around-time of specimens, and much more. We take a “We Are One *Championship* Team” attitude with all our physician clients including their staff and an approach to patient care that is unsurpassed in our industry.

Leadership Team

Dr. David Baunoch – Founding Partner

As Founding Partner, Dr. David Baunoch is actively involved in the development of healthcare innovation, operationalizing market-focused concepts and managing Echelon companies. Dr. Baunoch serves as CEO of two Echelon companies, and is the Founder of Transpera Diagnostics and Combimatrix Molecular Diagnostics.

With over 25 years of laboratory experience, Dr. Baunoch has held a variety of executive management positions with industry-leading companies and institutions. He served as Vice President of Lab Operations and Research and Development for US LABS, being a part of the team that developed the first TC/PC model. He was Director of Marketing at Ventana Medical Systems, Assistant Professor in the Department of Pathology, Director of the Immunohistochemistry and Special Procedures Section at The University of Chicago Hospitals, and Director of the Immunohistochemistry Core Laboratory at The University of Chicago Comprehensive Cancer Research Center. Dr. Baunoch received his PhD in Microbiology and Molecular Genetics from Wayne State University and completed a NIH Postdoctoral Fellowship in Breast Cancer Research at the Michigan Cancer Foundation. Dr. Baunoch has been involved in the development of over 500 laboratory developed tests and holds a number of patents for diagnostics tests and instrumentation. He has served on the faculty of several colleges and universities, and is widely published, with over 25

David Pauluzzi - Founding and Managing Partner

As Managing Partner, David Pauluzzi guides the strategic plan for the firm, oversees the development of the Echelon portfolio, and manages the operations of the firm.

Mr. Pauluzzi has a strong track record of building and growing businesses during a distinguished career spanning nearly 30 years in healthcare. He has led diagnostic and healthcare services companies ranging in size from $10 million to $1 billion in revenues. These included publicly traded corporations, and start-up or mid-sized companies backed by private equity or venture capital firms. Prior to launching Echelon, Mr. Pauluzzi was president and CEO of PLUS Diagnostics

(now Miraca Life Sciences), an anatomic pathology laboratory. He also served as Executive Director/Vice President with Quest Diagnostics, the world's leading provider of diagnostic information services, Vice President of Sales and Marketing/COO with US Labs, and he held positions in sales and marketing for Ventana Medical Systems (now Roche Diagnostics), a medical device and reagent business. Mr. Pauluzzi began his career with Abbott Laboratories, spending nine years in various roles in their diagnostics division. He received his bachelor's degree in public accounting from Loyola University of Chicago, and is a member of the CEO Roundtable at the University of California-Irvine.

Kevin Mannix - Partner and Chief Commercial Officer

As Chief Commercial Officer, Kevin Mannix is responsible for the firm's market strategy and development, including sales, product development and customer relations to drive business growth and market share.
Mr. Mannix is Founder and CEO of Vested Cellutions and also the Founder and CEO of Stem Cellutions, a sales and marketing firm for Cord/Adult Stem Cells. With more than 23 years of experience with Baxter Healthcare / Dade Behring, Mr. Mannix has held several management positions with these medical companies including Executive Territory Sales Manager in there Laboratory Division. He was Founder and CEO of Long Island Laboratory and served as Director of Sales for NeoStem, an adult stem cell collection and storage company based in New York, NY.

The fourth of six articles providing an overview of UTIs

4. Management of urinary tract infections in the elderly

ANDREA COVE-SMITH AND MICHAEL ALMOND

Urinary tract infection is a common cause of morbidity and mortality in patients over 70 years of age. The prevention, diagnosis and treatment of infection in this group of patients require an understanding of the different epidemiology and pathophysiology involved.

Dr A. Cove-Smith, BA, BM BCh, MRCP, Specialist Registrar in Nephrology; **Dr M.K. Almond,** BMedSci, BM MS, DM, FRCP, Consultant Nephrologist, Renal Unit, Southend General Hospital, Essex.

It is well established that the prevalence of urinary tract infection (UTI) increases with age and this increase is seen in both sexes. In younger populations there is a strong female predominance, with a 50:1 female to male ratio, while in patients over 70 years of age the ratio is around 2:1. The annual incidence of UTIs in the elderly is around 10 per cent and it may be as high as 30 per cent for people living in nurs-ing homes and other institutions.[1] The mortality rate in bac-teraemic elderly patients with UTI is as high as 33 per cent;[2] prevention and early recognition is therefore essential.

Pathogenesis

Many factors contribute to the increased frequency of UTIs in the elderly. Increased colonisation of the skin with Gram-negative organisms occurs with increasing age and debilitation. These colonising organisms are then more likely to cause infection when other contributing factors become involved.

In postmenopausal women, the intravaginal pH is high and this is associated with a change in colonising organisms and increased bacterial adherence to the uro-epithelium (Figure 1).

In both men and women, the postvoiding residual volume increases with age. This is partly a result of changes in pelvic musculature, as well as bladder function in women and prostatic hypertrophy in men. It is exacerbated in some cases by dementia, neuromuscular disorders and the con-comitant use of anticholinergic drugs.

Increased instrumentation and decreased host defence mechanisms also contribute to the increased risk of elderly patients developing sepsis originating from the urinary tract.

Organisms – the usual suspects?

A wider variety of organisms are responsible for infections in the elderly than in younger patients. *Escherichia coli* is still the most common, causing 70 per cent of infections in uncatheterised patients. In patients with catheters or living in institutions, however, it is found in only 40 per cent of cases.[3] In these patients, other Gram-negative organisms such as *Klebsiella*, *Proteus* and *Pseudomonas* become more important, and some Gram-positive organisms such as ente-rococci and staphylococci are also more prevalent. Some series have found polymicrobial infections in as many as one-third of cases in the elderly. Fungi also become more prevalent with increasing age and with frequent antibiotic use. Knowledge of likely organisms and of local resistance patterns is crucial in determining appropriate empirical antibiotic treatment in these patients.

Figure 1. Atrophy of the vaginal epithelium leads to alteration in the spectrum of colonising organisms and increased susceptibility to urinary tract infections. (Courtesy of Mrs Patricia Wilson.)

Presenting symptoms

Elderly patients more commonly present with atypical or non-specific symptoms, and this may contribute to delayed diagnosis and treatment (Box 1). They still often present with the classic symptoms of dysuria, fever and frequency, which are seen in younger people, but they may have much more vague presentations such as an acute confusional state, decreased mobility or newly developed urinary incon-

tinence. It is important to recognise that UTI is only one possible cause of these atypical presenting symptoms. It is therefore essential to assess the patient fully for other possible diagnoses and to send a urine sample to confirm the diagnosis and guide antibiotic therapy.

Box 1. Symptoms of urinary tract infection in elderly patients.

Typical symptoms	*Atypical symptoms*
• Fever	• Altered mental state
• Dysuria	• New-onset incontinence
• Loin pain	• Nausea and vomiting
• Frequency	• Urinary retention

Prevention of UTIs

Urinary catheters

One of the most important preventive strategies in the elderly is to minimise the use of urinary catheters. Indwelling catheters are a major risk factor for UTI and subsequent sepsis. Bacterial colonisation is almost universal once catheters have been *in situ* for more than a few days; even with a closed drainage system, 50 per cent of catheters will be colonised within four days. In hospitalised patients, indwelling catheters are implicated in 40–75 per cent of all hospital-acquired UTIs.[4] Many strategies have been used to attempt to minimise colonisation and subsequent infection. Disinfecting the skin regularly and using disinfectants in the collecting system make no difference. There has been interest recently in the use of catheters with silver linings or impregnated with antimicrobials, but no convincing benefit has been demonstrated for long-term catheterisation.[5]

Careful catheter care and maintaining the integrity of a closed system can reduce infection rates, but the best strategy is to avoid catheterisation, unless absolutely necessary, and to practise early removal of any catheters that have been inserted (Box 2). Patients with outflow tract obstruction may require catheterisation pending a more permanent solution to the problem, such as transurethral resection of the prostate in benign prostatic hypertrophy. Patients with urinary incontinence should be properly assessed for any reversible cause before considering catheterisation, which should be seen as a last resort. In one American study, only 27.5 per cent of catheterised patients in a nursing home had been adequately evaluated for the cause of their incontinence.[6] Intermittent self-catheterisation should be considered in patients with atonic or neuropathic bladders as an alternative strategy that has lower infection rates.

Oestrogens

Topical oestrogen therapy in postmenopausal women has been shown to lower intravaginal pH and allow an increase in colonisation with lactobacilli, which replace the more pathogenic Gram-negative colonising organisms. A trial in 1993 showed a significant reduction in UTIs with oestrogen therapy in a group of postmenopausal women with recurrent infections.[7] The most convincing benefit seems to be with vaginal administration, and topical treatment is generally well tolerated. Topical oestrogen therapy should therefore be considered in older women presenting with recurrent UTIs, especially once other underlying factors such as diabetes mellitus and urinary tract abnormalities have been ruled out. Oral oestrogens have been shown to reduce postvoiding residual volumes, which may also reduce the frequency of UTIs,[8] but their benefit is yet to be proven, so their role remains unclear.

Cranberry juice

Cranberry juice has long been thought to help prevent or even treat UTIs, and several studies have assessed the effectiveness of this treatment. A few small studies have had promising results, but a review of seven trials found that, overall, the results are still equivocal.[9] As this is a very safe intervention and may have some benefit, it is reasonable to recommend cranberry juice to patients who wish to try it.

Treatment of asymptomatic bacteriuria

Asymptomatic bacteriuria is a common finding in elderly patients. Up to 10 per cent of men and 20 per cent of women over 80 years of age living in the community will have asymptomatic bacteriuria. These figures rise to 15–40 per cent and 25–50 per cent, respectively, in people in residential care facilities.[10] There is no evidence that screening for or treating asymptomatic bacteriuria routinely is beneficial in the elderly; indeed, it is likely to lead to an increase in antibiotic resistance, making future infections more difficult to treat. The only time that treatment of asymptomatic bacteriuria should be undertaken in the elderly is before instrumentation of the urinary tract to prevent systemic infection.

Box 2. Strategies to minimise catheter-related urinary infections.

- Ensure integrity of closed drainage system
- Early catheter removal
- Intermittent self-catheterisation where possible
- Smaller bore catheters
- Optimal hygiene technique when inserting catheter
- Consider silver-lined or antibiotic-impregnated catheters for short-term use (less than three weeks)

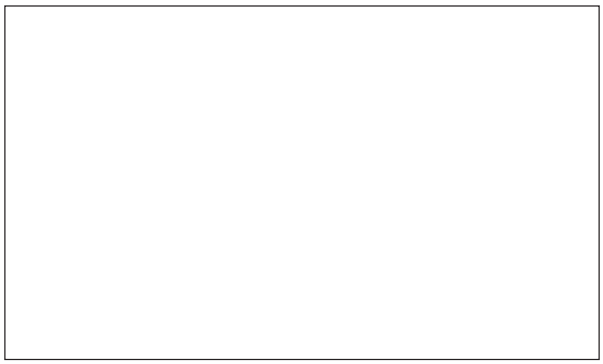


Figure 2. Skin reaction to ammonia in an elderly woman with longstanding urinary tract infection. (Courtesy of Mrs Patricia Wilson.)

Treatment of UTIs

Antibiotic therapy

Choosing the correct antibiotic is more complex in the eld-erly than in otherwise healthy young adults. The range of pathogens is larger and the potential for antibiotic resist-ance is higher, especially in patients who are institution-alised or who have had frequent courses of antibiotics (Figure 2). Other factors are the higher incidence of asso-ciated co-morbidity, in particular the increased frequency of renal insufficiency, and the concomitant use of addi-tional medication. It is also important to avoid unnecessary antibiotic courses and to use narrow-spectrum agents when-ever possible to minimise the risk of diarrhoea as a result of infection with *Clostridium difficile,* which causes signifi-cant morbidity and mortality in elderly populations.

Resistance to amoxicillin is high among isolates of *E. coli* in the UK (48.7 per cent); therefore it is not a good option for first-line therapy. Co-amoxiclav will cover 78.8 per cent of *E. coli* isolates and more than 95 per cent are sensitive to nitrofurantoin and cefuroxime. **However, it is important to remember that, although *E. coli* is the most common pathogen, other organisms are responsible for around half of UTIs in the elderly.** Susceptibility to trimethoprim ranges from 58.1 to 84.5 per cent over the range of common organisms. Ciprofloxacin covers 88.6–97.7 per cent of all likely pathogens and has the advan-tage of pseudomonal cover.[11]

The best practice for a suspected, mild, lower UTI is to send a urine sample for culture and sensitivity and not to start antibiotics until the result is available in order to avoid unnecessary use of broad-spectrum empirical treatment. This is possible only if the patient is systemically well and it is therefore safe to wait for further information. If empiri-cal treatment is required, it is important to look at the sen-sitivity pattern of any previous isolates, along with local resistance patterns and any recent therapy. A pre-therapy midstream urine sample should be sent and the antibiotic choice reassessed at 48–72 hours in light of the culture result.

Trimethoprim is a reasonable empirical agent for uncomplicated lower UTIs. For acute pyelonephritis, ciprofloxacin or second- or third-generation cephalosporins are recommended as first-line empirical therapy. Aminoglycosides can be used and are effective against most Gram-negative organisms, but drug monitoring must be performed, especially in the elderly. The culture and sensitivities will usually be available within 48 hours and the toxicity of aminoglycosides over this period is minimal, providing there is no renal impairment. Once sensitivities are known, the antibiotic regimen should be changed appropriately.

Duration of treatment

Acute pyelonephritis should be treated for 14 days with an antibiotic that is excreted in high concentrations in the urinary tract and covers the isolated organism. Acute pyelonephritis in elderly patients will often require hospital admission and parenteral therapy in the first instance. Prostatitis occurs not infrequently in elderly men and requires at least four weeks of an antibiotic that has good tissue penetration, such as ciprofloxacin or co-trimoxazole.[12]

The duration of antibiotic therapy in uncomplicated lower UTIs in the elderly is still debated. A double-blind randomised controlled trial conducted in 2004 showed that a three-day treatment course of antibiotic was not inferior to a seven-day course (98 and 93 per cent eradication rates, respectively) and the shorter course was better tolerated.[13] The seven-day course led to more frequent problems with drowsiness, headache, loss of appetite, nausea and vomiting.

Patients living in care homes are more likely to have com-plicating factors (such as incontinence, incomplete voiding and reduced immunity), and longer courses may be required to achieve eradication. However, a comparison between eld-erly patients with UTI in nursing homes and those in the community found **that nursing home residents are more likely to receive excessive antibiotic doses and to experience adverse drug events.** They were 83 times more likely than the elderly patients in the community to receive a prolonged course (seven days or more) and to receive re-treatment for persistent or recurrent symptoms.[14]

This suggests that care must be taken not to overtreat these patients but to review their clinical state after a few (three to five) days of therapy. Failure to respond to a short course should prompt a search for underlying problems such as diabetes mellitus, structural renal tract abnormal-ity or antibiotic resistance. Following a course of therapy, consideration should be given to demonstrating eradica-

tion in patients with renal tract abnormalities or in those who have a history of recurrent UTIs.

Dose adjustment

Careful attention should be paid to appropriate dosing in the elderly. Renal impairment is common in this age group and often unrecognised, as a serum creatinine concentration within the 'normal' range may represent a significantly reduced glomerular filtration rate (GFR) in an elderly woman. Nursing home residents, in particular, are more likely than patients in the community to have unrecognised renal impairment, which should prompt dose adjustment.[14] There has been a significant increase in routine reporting of calculated GFR from biochemistry laboratories following the recent publication of the national service framework for renal services.[15] This will allow doctors to have easy access to a more accurate measurement of renal function and therefore to use the appropriate dose adjustment by referring to Appendix 3 in the *British National Formulary*.

Other measures

Hospitalised patients who have been catheterised and who develop a catheter-associated UTI should have their catheter removed as soon as possible. Patients with long-term urinary catheters should have the need for their catheter reassessed. If the indication is unclear, it could be removed and not replaced and the patient monitored for urinary retention or problematic incontinence. This would provide the opportunity to consider other potential solutions and hopefully avoid further catheter-related problems. If long-term catheterisation is definitely required and alternatives have been ruled out, most people would advocate changing the catheter during the course of antibiotic treatment. If the current indwelling catheter is blocked, it should be changed as soon as possible under antibiotic cover.

All elderly patients who develop a UTI should be investigated for diabetes mellitus, as this is a simple diagnosis to exclude and an important one not to miss. A basic history and examination should pick up problems with urinary incontinence or obstructive symptoms. Anyone with recurrent UTIs or symptoms suggestive of bladder outflow problems should have a pre- and postmicturition ultrasound scan of the kidneys, urinary tract and bladder. This would assist in excluding urological problems associated with an increased frequency of UTIs or a difficulty with eradication; renal or bladder stones, tumours and other anatomical anomalies.

References

1. Porush JG, Faubert PF, eds. *Renal disease in the aged*. Boston, MA: Little Brown, 1991; 241-58.
2. Tal S, Guller V, Levi S, *et al*. Profile and prognosis of febrile elderly patients with bacteraemic urinary tract infection. *J Infect* 2005; **50**(4): 296-305.
3. Rajagopalan S. Urinary tract infections. In: Beers MH, Jones TV, eds. *Merck manual of geriatrics*, 3rd edn, section 12, ch. 100. Merck Publishing, 2000.
4. Wilkie ME, Almond MK, Marsh FP. Diagnosis and management of urinary tract infection in adults. *BMJ* 1992; **305**: 1137-41.
5. Maki DG, Tambyah PA. Engineering out the risk of infection with urinary catheters. *Emerging Infectious Diseases* 2001; **7** (Special Issue): 1-6.
6. Ribeiro BJ, Smith SR. Evaluation of urinary catheterisation and urinary incontinence in a general nursing home population. *J Am Geriatr Soc* 1985; **33**: 479-82.
7. Raz R, Stamm WE. A controlled trial of intravaginal estriol in postmenopausal women with recurrent UTIs. *N Engl J Med* 1993; **329**: 753-6.
8. Stern JA, Hsieh YC, Schaeffer AJ. Residual urine in an elderly female population: novel implications for oral estrogen replacement and impact on recurrent urinary tract infection. *J Urol* 2004; **171** (2 Pt 1): 768-70.
9. Jepson RG, Mihaljevic L, Craig J. Cranberries for preventing urinary tract infections. *Cochrane Database Syst Rev* 2004; **2**: CD001321.
10. Nicolle LF. Asymptomatic bacteriuria. When to screen and when to treat. *Infect Dis Clin North Am* 2003; **17**(2): 367-94.
11. Farrell DJ, Morrissey I, De Rubeis D, *et al*. A UK multicentre study of antimicrobial susceptibility of bacterial pathogens causing UTI. *J Infect* 2003; **46**(2): 94-100.
12. Lipsky BA. Prostatitis and urinary tract infection in men: what's new; what's true? *Am J Med* 1999; **106**(3): 327-34.
13. Vogel T, Verreault R, Gourdeau M, *et al*. Optimal duration of antibiotic therapy for uncomplicated urinary tract infection in older women: a double-blind randomised controlled trial. *CMAJ* 2004; **170**(4): 469-73.
14. Takahashi P, Trang N, Chutka D, *et al*. Antibiotic prescribing and outcomes following treatment of symptomatic urinary tract infections in older women. *J Am Med Dir Assoc* 2004; **5** (2 Suppl): S11.
15. Department of Health. National Service Framework for Renal Services. *Estimating glomerular filtration rate (eGFR): information for general practitioners*, 2006. www.dh.gov.uk

KEY POINTS

- Urinary tract infections increase in prevalence with increasing age and, unlike in younger adults, the incidence is similar between the sexes.
- Older patients are more likely to present with non-specific symptomatology.
- Prevention is paramount – limit use of indwelling urinary catheters; consider oestrogen therapy in postmenopausal women.
- Choose appropriate antibiotics according to local sensitivity patterns and previous isolates; narrow the spectrum as soon as possible.
- Avoid prolonged courses of antibiotics in simple UTIs in women and adjust the dose if indicated in view of increased toxic side-effects in elderly populations.
- Consider excluding primary causative pathology, diabetes and urological disease.

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Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems

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Abstract

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Urinary tract infections (UTIs) are common in pregnant women and pose a great therapeutic challenge, since the risk of serious complications in both the mother and her child is high. Pregnancy is a state associated with physiological, structural and functional urinary tract changes which promote ascending infections from the urethra. Unlike the general population, all pregnant women should be screened for bacteriuria with urine culture, and asymptomatic bacteriuria must be treated in every case that is diagnosed, as it is an important risk factor for pyelonephritis in this population. The antibiotic chosen should have a good maternal and fetal safety profile. In this paper, current principles of diagnosis and management of UTI in pregnancy are reviewed, and the main problems and controversies are identified and discussed.

Keywords: pregnancy, asymptomatic bacteriuria, acute cystitis, acute pyelonephritis

Introduction

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Urinary tract infections (UTIs) in pregnant women continue to pose a clinical problem and a great challenge for physicians. Although the incidence of bacteriuria in this population is only slightly higher than in non-pregnant women, its consequences for both the mother and the unborn child are more severe. There is a much higher risk (up to 40%) of progression to pyelonephritis, and possibly increased risk of pre-eclampsia, premature birth and low neonatal birth weight [1–6]. That is related to profound structural and functional urinary tract changes, typical for pregnancy. In about 80% of pregnant women dilation of the urinary tract combined with slight hydronephrosis is observed, caused partly by a reduction in smooth muscle tone with slowing of ureteral peristalsis, and partly by urethral

sphincter relaxation. This may be due to high levels of circulating progesterone [1, 7]. Simultaneously, the enlarged uterus compresses the urinary bladder, thus increasing the intravesical pressure, which may result in vesico-ureteral reflux and urine retention in the bladder after miction, commonly observed in pregnant women. Urinary stasis and impairment of the physiological anti-reflux mechanism create conditions favorable for bacterial growth and ascending infection. The additional predisposing factors include pregnancy-specific biochemical changes in urine, with higher amounts of glucose, amino acids and hormone degradation products, which increase urinary pH [7, 8].

Similarly as in non-pregnant women, in pregnant women UTIs are classified either as asymptomatic bacteriuria (ASB), when the infection is limited to bacterial growth in urine, or symptomatic infections (acute cystitis, acute pyelonephritis), when bacteria invade urinary tract tissues, inducing an inflammatory response. The UTIs in pregnancy are by definition considered complicated infections and require a special diagnostic approach and management.

Epidemiology and risk factors

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Urinary tract infections remain among the most common medical complications during pregnancy. It is estimated that the prevalence of ASB varies between 2% and 10–13%, similar to nonpregnant women [9–13]. There is a scarcity of data concerning acute cystitis in pregnancy; according to the available studies it is observed in 1–4% [11, 14, 15]. The prevalence of acute pyelonephritis in most reports ranges from 0.5% to 2% of pregnancies [1, 8, 16, 17].

Many women acquire bacteriuria before pregnancy [18, 19]. A large retrospective analysis with logistic regression modeling, embracing 8037 women from North Carolina, revealed that the two strongest predictors of bacteriuria at prenatal care at prenatal care initiation were: UTI prior to prenatal care initiation (OR = 2.5, 95% CI: 0.6–9.8 for whites, and OR = 8.8, 95% CI: 3.8–20.3 for blacks) and a pre-pregnancy history of UTI (OR = 2.1, 95% CI: 1.4–3.2) [19]. In a second analysis, prior antenatal UTI was found to be the strongest predictor of pyelonephritis after 20 weeks' gestation (OR = 5.3, 95% CI: 2.6–11.0) [20]. Other suggested risk factors for UTI during pregnancy are lower socioeconomic status, sexual activity, older age, multiparity, anatomical urinary tract abnormalities, sickle cell disease and diabetes, although the significance of some of them (age, parity or sickle cell trait) remains a matter of controversy [1, 10, 21–23].

Microbiology

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The pathogens responsible for infections during pregnancy are similar to those in the general population. Most infections are caused by *Enterobacteriaceae*, commonly found in the gastrointestinal tract, with *Escherichia coli* responsible for 63–85% of cases, and among the remaining: *Klebsiella pneumoniae* (~8%), coagulase-negative *Staphylococcus* (up to 15%), *S. aureus* (up to 8%), and group B streptococci (GBS) (2–7%) [16, 17, 24–26].

Consequences of urinary tract infection in pregnancy

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Asymptomatic bacteriuria

The only serious maternal consequence of untreated ASB in pregnant women is a significant risk of acute pyelonephritis in later pregnancy (30–40% vs. 3–4% in treated patients) [27].

The results of the studies on perinatal outcomes of untreated ASB are controversial. Although a number of them demonstrated a relationship of ASB in pregnant mothers and the risk of premature

delivery and/or lower birth weight, some other studies failed to prove the association [28–32]. The Cochrane Library meta-analysis revealed that antibiotic treatment was effective in reducing the incidence of low-birth-weight infants but not of preterm deliveries [27]. However, the authors stressed the poor methodological quality of the available studies, their different design, lack of sufficient information about the randomization methods, different definitions used, low statistical power and some substantial biases, urging caution in drawing conclusions. A good example of these problems is presented by the Cardiff Birth Survey [33]. In a prospectively studied large cohort of 25 844 pregnancies, several demographic, social and medical factors (including bacteriuria) were significantly associated with preterm birth in the initial univariable analyses. However, after adjustments for other medical factors, bacteriuria retained an association of only borderline significance, and after further adjustment for demographic and social factors, the relationship completely disappeared. The results of the second analysis of the same cohort, aimed to compare associations of studied factors with spontaneous vs. indicated preterm birth, are even more interesting [34]. Two separate multiple logistic regression analyses revealed that spontaneous and indicated preterm births have different overall profiles of risk factors, and only the last of them was associated with bacteriuria. The authors concluded that ASB, if it does not progress to symptomatic UTI, is not associated with preterm delivery.

Maternal GBS bacteriuria in a pregnant woman is considered a marker for genital tract colonization with this organism which poses a significant risk of preterm rupture of the membranes, premature delivery and early-onset severe neonatal infection [1, 24, 26, 35–37].

Symptomatic urinary tract infection

About 15–20% of women with pyelonephritis have bacteremia [8, 17]. They may develop various complications, such as acute kidney injury, anemia, hypertension, preeclampsia, sepsis and septic shock, hemolysis, thrombocytopenia, and acute respiratory distress syndrome, particularly if treatment is initiated too late [17, 27, 38–44]. Although these associations have not always been proved to be causal, most of the complications seem to be due to renal or other tissue damage caused by bacterial endotoxins and a systemic inflammatory response with endothelial injury [42, 45].

A number of observational studies have demonstrated the relationship between maternal symptomatic UTI and the risk of premature delivery and lower birth weight [28–30, 46]. The frequency of preterm deliveries in women with acute pyelonephritis is significantly higher than in women free of this complication, and pyelonephritis seems to be an important independent risk factor for delivery before 37 weeks' gestation [2, 5, 47]. However, again, a substantial heterogeneity between these studies, together with many possible biases, makes it difficult to establish the overall contribution of UTI to preterm birth [48]. A rare but severe complication is the transmission of the infection onto the newborn baby [49]. Very often the transmitted infection originates from a heavily colonized birth canal, usually with GBS [26].

Safety of antimicrobial treatment

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Nearly all antimicrobials cross the placenta, and some of them may exert teratogenic effects. Commonly accepted antibiotics used in treating UTIs during pregnancy, regardless of its period, include derivatives of penicillin and cephalosporins, particularly those with low protein-binding ability (such as cephalexin), all of FDA pregnancy category B (Table I) [50].

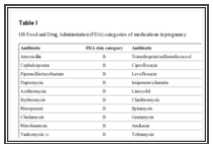

Table I

US Food and Drug Administration (FDA) categories of medication

Nitrofurantoin and trimethoprim/sulfamethoxazole should be avoided during the first trimester due to a possible risk of fetal defects, although the studies on that issue yield somewhat contradictory results [1, 51–53]. In the large American population-based National Birth Defects Prevention Study, maternal use of sulfonamides and nitrofurantoin (1 month before pregnancy to the end of the first trimester) was associated with more serious defects than any other antibacterial classes [51]. However, this study has been criticized for several significant limitations including recall bias (women were asked about antibiotic use after pregnancy and it was not confirmed by medical records), inability to determine whether the birth defect was due to the antibiotic itself, the infection for which the antibiotic was prescribed, or other confounding factors. Two years later, the Committee of Obstetrics Practice of the American College of Obstetricians and Gynecologists, summarizing the available data on the relationship between prenatal exposure to both antimicrobials and birth defects, concluded that: 1) "When selecting an antibiotic for a true infection during the first trimester of pregnancy (that is, during organogenesis), health care providers should consider and discuss with patients the benefits as well as the potential unknown risks of teratogenesis and maternal adverse reactions; 2) "Prescribing sulfonamides or nitrofurantoin in the first trimester is still considered appropriate when no other suitable alternative antibiotics are available"; 3) "Pregnant women should not be denied appropriate treatment for infections because untreated infections can commonly lead to serious maternal and fetal complications" [52]. Recently Nordeng *et al*. published the results from a large population-based cohort study using the Norwegian Prescription Database linked to data on all live births, stillbirths, and induced abortions after 12 weeks of gestation from the Medical Birth Registry of Norway [53]. Among 180 120 pregnancies between 2004 and 2008, 1334 women filled prescriptions for nitrofurantoin in the first trimester. The authors found that dispensing nitrofurantoin during the first trimester was not associated with increased risk of major malformations (OR = 0.79, 95% CI: 0.51–1.23) or higher rates of stillbirth, neonatal death, low birth weight, or preterm delivery.

In the second and third trimester, trimethoprim/sulfamethoxazole and nitrofurantoin are well tolerated and by some considered even first line agents, except in the last week before delivery, when they may increase neonatal jaundice and predispose to kernicterus [1, 10, 51–55]. The same concerns other antimicrobials with very high protein binding (e.g. ceftriaxone), since they can displace bilirubin from plasma proteins. One should also remember that trimethoprim (FDA pregnancy category C) is a folic acid antagonist; thus, supplementation of this agent and monitoring of its serum concentration are required during treatment [56, 57]. Nitrofurantoin can be theoretically associated with a risk of fetal or neonatal hemolytic anemia if the mother has glucose-6-phosphate deficiency, and although this complication in pregnancy has not been reported, the drug should be used with caution, particularly in areas of disease prevalence [10, 58, 59].

The use of fluoroquinolones (FDA pregnancy category C) is essentially contraindicated throughout pregnancy, since fetal cartilage development disorders have been reported in experimental animals, although not in human studies [27, 60–63]. In the largest study so far, 200 pregnant women exposed to fluoroquinolones were compared to 200 women exposed to nonteratogenic, nonembryotoxic antimicrobials, matched by indication, duration of therapy (~3 days), and trimester of exposure [60]. The rate of major congenital malformations did not differ between the group exposed to quinolones in the first trimester and the control group (2.2% vs. 2.6%; RR = 0.85; 95% CI: 0.21–3.49) and was

within the expected normal range (1–5%). A systemic review of prospective, controlled studies showed that the use of fluoroquinolones during the first trimester of pregnancy does not appear to be associated with an increased risk of major malformations recognized after birth, stillbirths, preterm births or low birth weight [64]. Apparently more data are needed to establish safety of fluoroquinolones in pregnancy before they may be routinely prescribed. However, in some cases of complicated symptomatic UTI, resistant to other antibiotics, their benefits may outweigh the risks [60].

Gentamicin and other aminoglycosides are FDA pregnancy category D, because of their potential nephro- and neurotoxicity (eighth nerve damage) to the fetus [1, 65, 66]. Tetracyclines lead to discoloration of deciduous teeth if given after 5 months' gestation [65, 66]. Macrolides have been assigned to pregnancy category C by the FDA (Table I). Although these drugs are used in pregnancy relatively often, the data on their embryotoxicity and teratogenicity are limited. Earlier reports suggested an association between prenatal exposure to macrolides and congenital heart defects or pyloric stenosis, whereas the results of some recent studies are rather reassuring [51, 67–71]. The first prospective controlled multicenter study of exposure to clarithromycin in early pregnancy suggested that this agent does not increase the risk of fetal malformations above the expected 1–3% [68]. However, there was a two-fold higher rate (14% vs. 7%) of spontaneous abortions in the exposed vs. control group, and although it still remained within the expected baseline rate, the possibility that it could be a result of undiagnosed fetal malformation cannot be excluded. No significant teratogenic effect of erythromycin was identified in a Hungarian case-control study, a nationally based registry of cases with congenital abnormalities [69]. The main limitations of this data set were: a relatively low response rate, retrospective collection of data (recall bias), inability to exclude the effect of other drugs, and a restriction of the study to the second and third trimester. However, in a large prospective observational study, performed in 511 women exposed to macrolides during the first trimester, Bar-Oz *et al*. did not observe a significant difference in the rate of major congenital malformations between the study group and comparison group [70]. Recently, Lin *et al*. compared the prenatal usage of erythromycin and nonerythromycin macrolides by mothers of 4132 infants with a congenital heart defect and 735 with pyloric stenosis and mothers of 6952 infants without any malformation, serving as a control group [71]. In logistic regression analysis they found no association of exposure to the drugs and increased risk of both types of birth defects. Again, these results should be interpreted with caution, since the power of the study was limited and – as the authors underline – modest associations could be missed. So further studies are needed before the macrolides become accepted for wide use. Until then, this group of antibiotics should be reserved for the treatment of serious or life-threatening conditions, unresponsive to standard antibiotic therapy.

Interesting findings came from the ORACLE Children Study II, which assessed the long-term outcomes for 3190 children born to women who had received antibiotics vs. placebo for threatened preterm labor with intact membranes [72]. The study sought follow-up information for children at age 7 in the UK using a parent-report postal questionnaire. The authors found that exposure to erythromycin or amoxicillin-clavulanate significantly increased the number of children with various functional impairments and cerebral palsy compared to placebo (OR = 1.42, 95% CI: 0.68–2.98, and OR = 1.22, 95% CI: 0.57–2.62, respectively). The risk was greatest when both antibiotics were given together compared to double placebo (OR = 2.91, 95% CI: 1.50–5.65). The cause of this neurological dysfunction is unclear, but it could be a result of subclinical perinatal infection as well as a direct effect of the antibiotics on the fetal brain or cerebral blood flow. Alternatively the antibiotic might have negatively influenced microbial colonization of newborn children, with long-lasting consequences. There are some suggestions that antibiotics alter immune tolerance by changing the fetal gut flora, thus

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contributing to the substantial increase in the incidence of asthma, allergies, autoimmune diseases, autism, ADHD and other chronic conditions [73–75]. The main conclusion from all these interesting studies is that we should be very cautious in prescribing antibiotics to pregnant women in the absence of proven benefit (e.g. spontaneous preterm labor with intact membranes), while in situations clearly associated with increased risk of maternal, fetal and neonatal death (e.g. clinical signs of chorioamnionitis) antimicrobial therapy is necessary.

Diagnosis and treatment of different clinical forms of urinary tract infection

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The criteria for diagnosis and treatment of UTI are more restrictive compared with the general population, since the potential risks concern not only an expectant mother but also her unborn child.

Asymptomatic bacteriuria

Screening tests Given the evidence that effective antimicrobial therapy of ASB in pregnancy significantly reduces the risk of pyelonephritis and possibly also adverse fetal outcomes, routine screening for the presence of clinically significant bacteriuria in all pregnant women has become necessary. Urine culture remains the most reliable test allowing the diagnosis of ASB. According to recommendations developed by the IDSA (Infectious Diseases Society of America), significant bacteriuria in asymptomatic women is defined as bacterial monoculture in the quantity of $\geq 10^5$ colony-forming units (CFU) per ml in two consecutive mid-stream clean-catch urine specimens or $\geq 10^2$ CFU/ml in urine collected from single urinary bladder catheterization [76]. However, for practical and economic reasons the guidelines for routine screening in pregnancy accept a single urine culture taken between weeks 12 and 16, or at first prenatal visit (if later), although there is only an 80% probability that the woman has true bacteriuria (vs. 95% with the original criteria) [9, 77]. Due to this high rate of false positive results, in some centers women with a positive urine culture are asked to return within 1 week for the second testing, to avoid unnecessary treatment [11].

A question which remains unanswered is: should women in whom no ASB was detected upon the first examination have additional screening in later pregnancy? To date, repeated tests have been recommended only in high-risk women (with diabetes, sickle cell anemia, immunological defects, urinary tract abnormalities or a history of recurring infections before pregnancy) [2, 38]. However, the more recent reports suggest that repeating the urine culture in each trimester improves the detection rate of ASB [78, 79]. McIsaac *et al*. studied 1050 women who were subjected to successive urine cultures before week 20, at week 28 and at week 36 [78]. A total of 49 cases of ASB were detected (prevalence 4.7%). The authors demonstrated that basing the diagnosis on a single urine culture before 20 weeks' gestation leaves more than one-half of ASB cases undiagnosed, since 40.8% of diagnoses were made after the first culture vs. 63.3% after the second vs. 87.8% after the third culture. In a much smaller Turkish study, ASB prevalence distribution in the first, second, and third trimesters was 0.9%, 1.83%, and 5.6%, respectively [79]. That suggests that many women with no bacteriuria in their initial examination in the first trimester may develop bacteriuria during the later trimesters. The authors of these studies conclude that it would be prudent to screen pregnant women for bacteriuria also in the second and third trimesters [78, 79]. However, until large, prospective, randomized clinical trials (RCTs) are available and a clear benefit of this routine additional screening is observed, no recommendation can be made for or against it.

Treatment The presence of ASB in a pregnant woman is an absolute indication for initiation of the

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treatment. The benefits of such a strategy with bacteriological follow-up were summarized by Smaill and Vazquez for the Cochrane Library, on the basis of the results of 14 RCTs, embracing 2302 pregnant women with ASB, in which the effects of different antibiotics given for different duration were compared to placebo or untreated groups [27]. The analysis of 5 of these trials, involving 820 pregnancies, showed that antibiotics effectively cleared ASB (RR = 0.25, 95% CI: 0.14–0.48). In the same review the authors present the results of another analysis which included 11 trials and 1955 pregnancies, which demonstrated that antibiotic treatment of ASB may reduce the incidence of pyelonephritis by 52–86% (RR = 0.25, 95% CI: 0.14–0.48).

Management of ASB in pregnancy consists of short-term, usually 5–7 days, oral antibiotic therapy [76]. Basic principles of management are presented in Table II. In the face of the rapidly developing antibiotic resistance, the current position is that the treatment should be based on microbial sensitivity testing. Recently, a growing number of authors suggest that a reasonable first choice drug in the second and third trimester is the old and almost forgotten nitrofurantoin [80–82]. As shown by most recent studies, nitrofurantoin is active against nearly 90% of *E. coli* strains isolated from urine, including 89% of extended spectrum β-lactamase (ESBL)-producing strains [81, 82]. Kashanian *et al*. carried out a retrospective analysis of drug resistance among bacteria cultured from urine specimens in a single hospital in New York in 2003–2007 [81]. Out of 10 417 cultures in which *E. coli* growth was achieved, 95.6% were sensitive to nitrofurantoin, with the average resistance rate of 2.3%, being significantly lower than that of ciprofloxacin, levofloxacin and trimethoprim/sulfamethoxazole (24.2%, 24% and 29%, respectively). A single 3 γ dose of phosphomycin also has a low resistance rate in *E. coli* infections and seems to be effective in non-pregnant women, but there is limited experience in using this regimen in pregnancy, and until more data become available it should not be given [50].

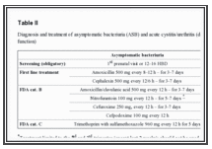

Table II

Diagnosis and treatment of asymptomatic bacteriuria (ASB) and acute cystitis/urethritis (doses for normal renal function)

Women with GBS isolated from the urine at any point during pregnancy should be treated according to the CDC (Centers for Disease Control and Prevention) guidelines, revised in 2010 and endorsed by the ACOG (American College of Obstetricians and Gynecologists) and AAP (American Academy of Pediatrics) [24]. In asymptomatic women with urinary colony counts < 100 000 CFU/ml, antimicrobial agents are not recommended before the intrapartum period, since such treatment is not effective in eliminating GBS carriage or preventing neonatal disease and can cause adverse consequence. Symptomatic UTI or GBS significant ASB should be treated according to current standards of pregnancy care [25, 77]. All of them (regardless of level of CFU/ml), at the time of labor or rupture of membranes, should receive appropriate intravenous antibiotics for the prevention of early-onset neonatal GBS disease, and do not need rescreening by genital or urinary tract culture in the third trimester, as they are presumed to be GBS colonized [24, 83, 84]. The same approach is recommended for women who had a previous infant with invasive GBS disease. All other patients should be screened at 35–37 weeks' gestation for vaginal and rectal GBS colonization [24, 84].

Follow-up urine cultures All pregnant women with ASB should have periodic screening after therapy, since as many as one third of them experience a recurrent infection [58, 76]. Follow-up cultures should be obtained 1–2 weeks after treatment and then repeated once a month [58, 76]. In case of persistent or recurrent bacteriuria, longer antibiotic therapy using the same agent (e.g. 7 instead of 3 days of treatment) or another first line drug is recommended. Subsequent treatment courses are administered

until the bacterial counts drop to non-significant levels [56]. If bacteriuria persists despite repeated courses of therapy, as well as in women with additional risk factors (e.g. immunosuppression, diabetes, sickle cell anemia, neurogenic bladder) or recurrent/persistent UTIs before pregnancy, one should consider antimicrobial prophylaxis [10, 56]. Patients with recurrences associated with sexual activity may be offered postcoital prophylaxis a single antibiotic dose (e.g. nitrofurantoin 50–100 mg *p.o.* or cephalexin 250–500 mg *p.o.*) postcoitally [56, 85]. The remaining women may be given small doses of antibacterial agents (e.g. nitrofurantoin 50–100 mg in the evening) until the end of the pregnancy. In this group the follow-up urine culture is performed only at the beginning of the third trimester. In case of significant bacteriuria, prophylactic doses should be replaced by another course of antimicrobials, based on susceptibility testing [56].

Cystitis/urethritis

Diagnosis The diagnosis is made on the basis of symptoms (cloudy urine, dysuria, frequency, urgency, abdominal or suprapubic pain) and the presence of even small bacterial colony counts ($\geq 10^2$–10^3 CFU/ml) [56].

Management In most cases of lower UTI, the treatment is similar to that used in ASB (Table II) and should be guided by antimicrobial susceptibility testing. The optimal duration of treatment is unknown, but longer courses (5–7 days) of the therapy are generally suggested [12, 55, 58, 86]. Follow-up urine cultures are recommended 1–2 weeks after the treatment and then once a month. In women receiving chronic immunosuppression, management discussed in the section on ASB should be followed. In women with recurrent acute cystitis, antimicrobial urinary suppression based on daily use of a small dose of antibacterial drug during the symptom-free period is recommended or, in the case of an evident relationship of the disease with sexual activity, only after intercourse (e.g. nitrofurantoin 50–100 mg, cephalexin 250–500 mg) [56, 85].

Acute pyelonephritis

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Acute pyelonephritis is most common in late pregnancy, with 80–90% of cases occurring in the second and third trimester [16, 17, 29, 38, 87]. It is usually a consequence of undiagnosed or inappropriately treated lower UTI, or a complication of 30–40% of cases of untreated ASB [8]. The overall incidence of pyelonephritis reaches up to 2% of all pregnancies (vs. < 1% in the general population [2, 8]. Besides ASB, the other risk factors of acute pyelonephritis include: mother's age, nulliparity, sickle cell anemia, diabetes, nephrolithiasis, illicit drug use, history of pyelonephritis and maternal urinary tract defects [2, 8, 24, 58].

The clinical presentation is typical and includes lumbar pain, fever of > 38°C, chills, nausea, vomiting and costo-vertebral angle tenderness, while symptoms of dysuria are less common. Nearly one in five of pregnant women with pyelonephritis has septicemia at diagnosis [3, 8, 17, 40]. Hill *et al*. among 32 282 pregnant women, who had been admitted to their prenatal clinic during the 2-year study period, identified 440 cases of pyelonephritis [17]. Complications included: anemia (23%), septicemia (17%), transient renal dysfunction (2%), and pulmonary insufficiency (7%). Numbers of preterm births and small-for-gestational-age infants were not increased as compared with expected rates in this hospital.

Management

Basic principles of management are presented in Table III. According to the 2005 IDSA guidelines, all suspected cases of pyelonephritis should be hospitalized at least for the initial 48 h of treatment [76].

However, some authors believe that in carefully selected cases, when a definite diagnosis of pyelonephritis can be made and a strict medical follow-up is possible, outpatient treatment may be attempted [38, 88, 89]. This is the case mostly in young, hitherto healthy women before 24 weeks' gestation and free of severe symptoms such as high fever with chills, persistent vomiting, significant dehydration or clinical signs of sepsis (tachycardia, tachypnea or hypotension) [38, 39, 49]. It should be stressed, however, that these are only opinions, based mostly on observational and a few small RCTs, and that in contrast to the overall population, evidence regarding the safety of such management is not available [89–91]. Appropriate hydration of the patient is a very important part of the treatment regardless of the setting. Beside urine and blood culture, recommendations include basic laboratory analyses (complete blood counts, electrolytes, creatinine, liver parameters, coagulation profile) and an ultrasound scan, which usually reveals dilation of pyelocalyceal systems and allows exclusion of other causes of the symptoms (e.g. renal abscess, ureter obstruction, other abdominal infections).

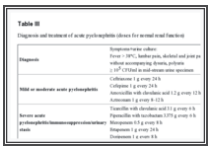

Table III

Diagnosis and treatment of acute pyelonephritis (doses for normal renal function)

In all patients, regardless of whether they are hospitalized, antibiotics should be given parenterally, for at least the first 48 h (until the resolution of fever). Usually the treatment is initiated empirically and verified after obtaining the microbial sensitivity test results [1, 76]. Forty-eight hours after resolution of symptoms, administration may be switched to the oral route. In case of fever persisting for more than 48 h, blood and urine cultures should be repeated, and any possible causes of treatment failure (perirenal abscess, lithiasis, congenital or acquired structural changes within the urinary tract) have to be carefully considered [38, 76]. Antibiotic therapy is usually continued for 10–14 days, although its optimum duration has never been established.

Unfortunately, there are not sufficient data available to recommend the specific treatment regimens in pregnant women. β-Lactam antibiotics are used most commonly, as they are relatively safe for the fetus (Table III). Carbapenems are reserved for the treatment of more severe cases, and those caused by multi-drug resistant bacteria. Administration of more toxic agents, such as aminoglycosides (potential fetal neuro- and nephrotoxicity) is acceptable only in cases when the expected benefits for the mother (e.g., in life-threatening conditions) outweigh the potential risk to the unborn child.

Recurrences of pyelonephritis, observed in 6–8% of pregnant women, pose a significant problem. In such cases, in periods free of symptoms, prophylactic treatment is recommended (e.g. nitrofurantoin 50–100 mg, cephalexin 250–500 mg before sleep), with urine culture at the beginning of the third trimester. Then upon detection of bacteriuria, prophylaxis is replaced by regular treatment [56]. In the aforementioned prospective study by Hill *et al*., after the successful treatment of pyelonephritis all studied women were placed on urinary suppression with nitrofurantoin, 100 mg daily, and were carefully followed up [17]. Only 12 of them (2.7%) were readmitted for recurrent pyelonephritis, and all 12 were found to be noncompliant with their antimicrobial suppression. However, again, this regimen is not supported by evidence obtained in RCTs. Recent analysis of the results of a randomized study that included 200 pregnant women showed no superiority of nitrofurantoin prophylaxis combined with standard of care (careful bacteriological control and antibiotic therapy upon detection of bacteriuria) over the standard of care alone [92].

Future challenges

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Due to the potential risk to mother and fetus, detection and effective treatment of UTIs remains an important clinical problem. It is advisable to assess risk factors for UTI in pregnancy, bearing in mind that some diagnostic procedures are not feasible and advisable to perform i.e. urodynamic studies [93]. Unfortunately, in contrast to the overall population, available data are scant, and the management guidelines were published several years ago and were largely opinion-based. The development of new recommendations requires well-planned, extensive studies, that would answer the still open questions regarding the frequency of screening and follow-up examinations, purposefulness of prophylaxis, safety of hitherto insufficiently studied or new antibiotics in pregnancy, and choice of optimum treatment regimens. If possible, any antibiotic use should be avoided in the first trimester, as this is the period of fetal organogenesis and nervous system development, with the highest risk of teratogenic effects of drugs.

Another disturbing problem, particularly in the aspect of fetal safety associated with therapeutic limitations, is the observed rapid development of antibiotic resistance. In general, this is applicable to diverse bacterial pathogens in many different clinical settings, and is becoming one of the most significant future threats to public health. In Gram-negative bacilli the resistance is associated with their ability to synthesize extended spectrum β-lactamases (ESBLs), as well as carbapenemases. The rapid spread of resistance is due to the fact that genes encoding β-lactamases and carbapenemases (particularly of the KPC type) are localized on mobile genomic elements (plasmids) easily transferable within the strain and among different strains of bacteria, even if the bacteria are not related to each other [94]. The introduction of new diagnostic methods with genetic typing may provide new opportunities in this area [95].

It is believed that currently more than a half of *E. coli* strains and more than one third of *Klebsiella* are ESBL+, leading to the resistance to third generation cephalosporins [15, 78, 80]. Enterobacteriaceae strains are resistant to all β-lactam and carbapenem antibiotics [94]. Another commonly observed phenomenon that has been known already for some years is meticillin resistance of Gram-positive cocci, which in practice often translates to multidrug resistance of these bacteria. One should also remember that antibiotic resistance of bacteria may differ depending on geographic area, hospital and even hospital ward, and the information on this topic may be crucial when making therapeutic decisions. Despite the diet in pregnancy is not generally different [96], we may think about some dietary approaches to change urinary pH as a prophylaxis of UTI in pregnancy

Conflict of interest

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The authors declare no conflict of interest.

References

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1. Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infection in pregnancy. Eur J Clin Invest. 2008;38(Suppl. 2):50–7. [PubMed]

2. Farkash E, Wientraub AY, Sergienko R, et al. Acute antepartum pyelonephritis in pregnancy: a critical analysis of risk factors and outcomes. Eur J Obstet Gynecol Reprod Biol. 2012;162:24–7. [PubMed]

3. Gravett MG, Martin ET, Bernson JD, et al. Serious and life-threatening pregnancy-related infections: opportunities to reduce the global burden. Plos Med. 2012;9:e1001324. [PMC free article] [PubMed]

4. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am

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