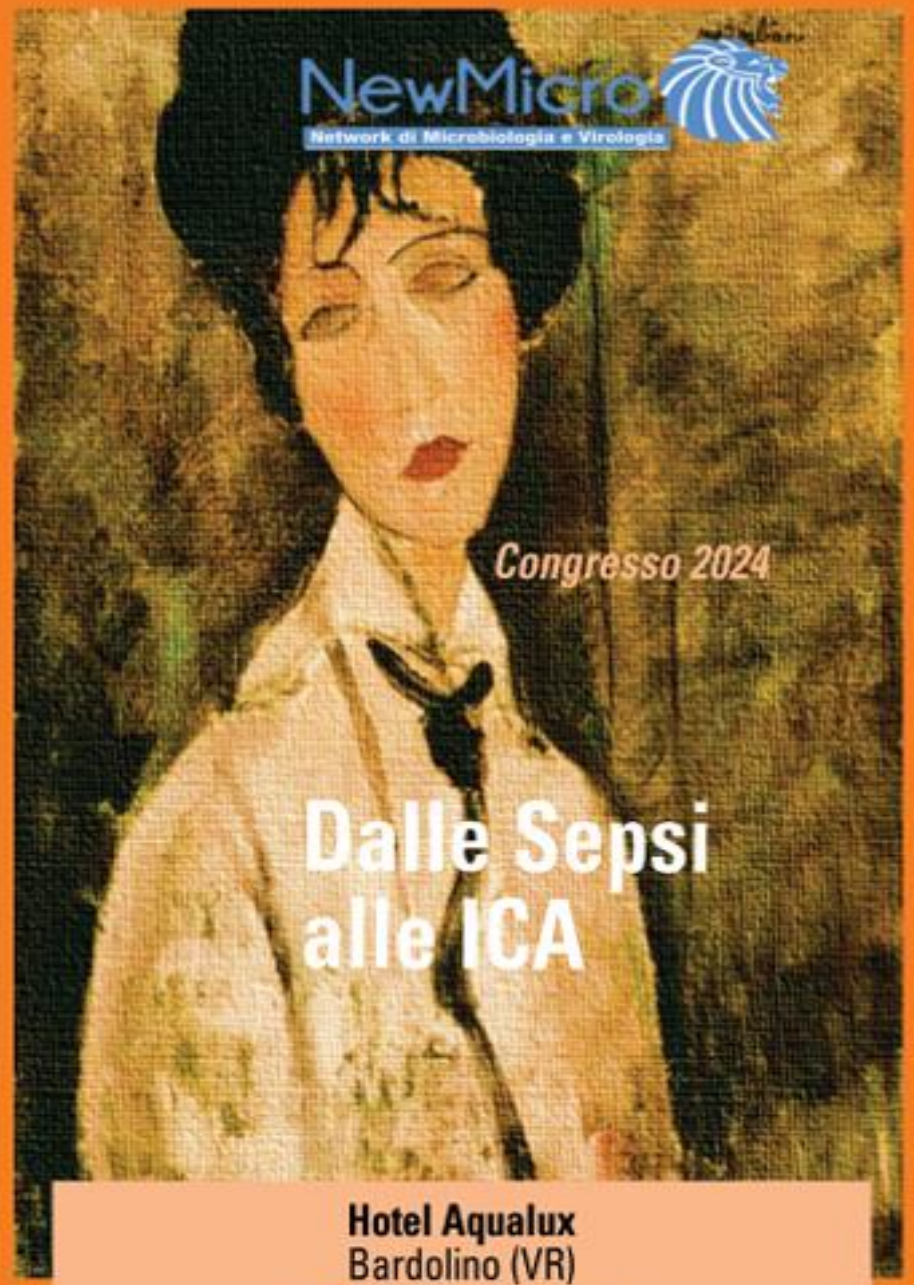
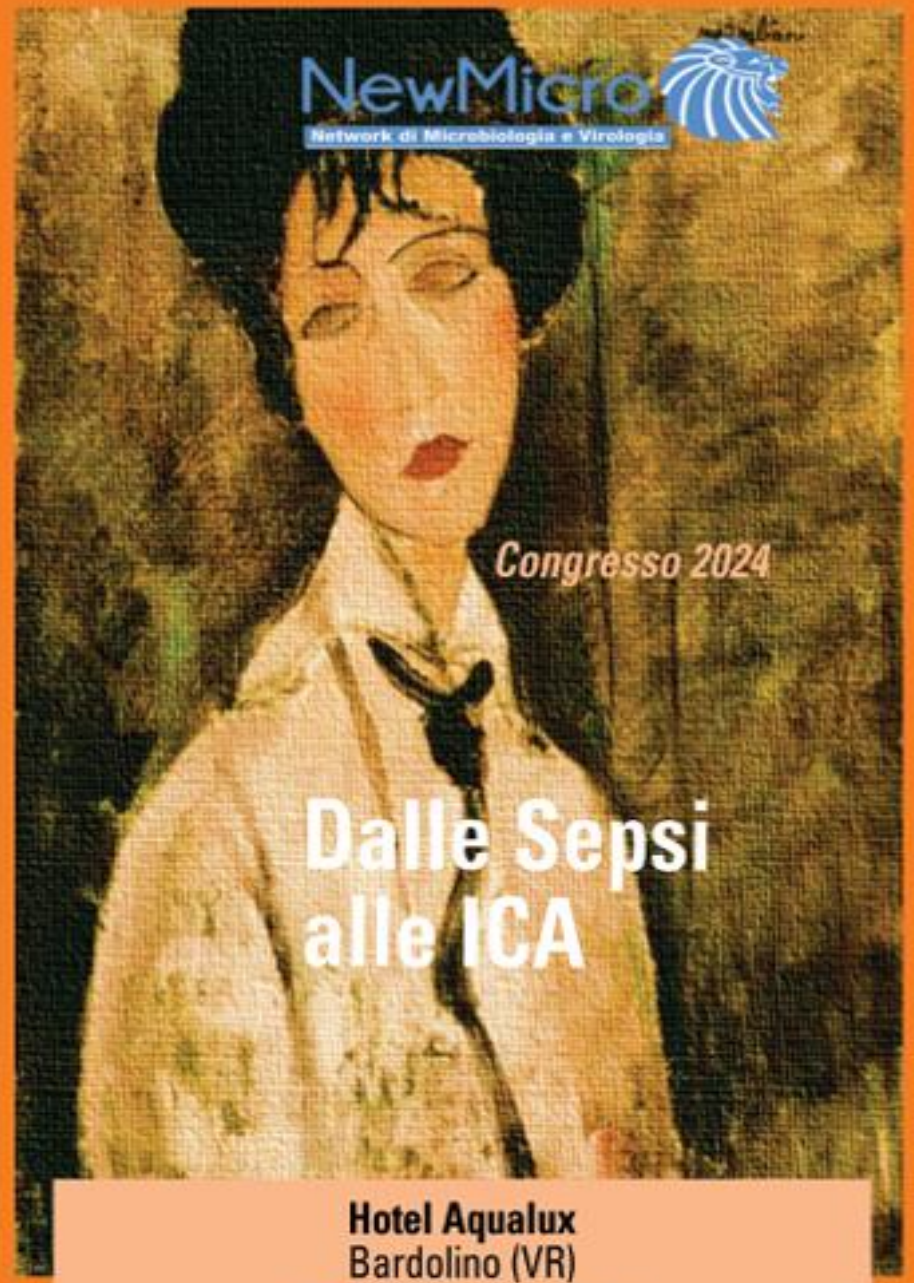


Atti del convegno

editors Giovanni Casiraghi & Marco Pradella

Armonizzazione e semantica del laboratorio nelle Sepsi ICA *Marco Pradella*
Il documento sulle Sepsi di Regione Lombardia *Maristella Moscheni*
Il sistema di sorveglianza di Regione Puglia *Viviana Vitale*
La prevenzione del rischio infettivo a garanzia della qualità dell'assistenza: "Sistema di Monitoraggio delle azioni regionali di controllo delle Infezioni Correlate all'Assistenza (SIMON)" *Paola M. Placanica*
Risvolti organizzativi correlati alle Sepsi ICA *Luca Fabbri - Annibale Raglio*
Infezioni delle vie urinarie *Fabio Manoni*
La risposta di una microbiologia alle calamità naturali *Vittorio Sambri*
Equità verticale, ICT e Sistemi Sanitari. Alcune applicazioni in Sanità *Fabrizio Clemente*
"La sfida ICA Sepsi: collaborazione tra medicina di laboratorio e clinica" *Graziella Bonetti, Andrea Patroni*
Sepsi, ICA e Infezioni Ossee *Tudor Draghici*
"Progetto Pedianet" *Elisa Barbieri*
Stewardship e TDM antimicrobici, due facce della stessa medaglia? *Ines Bianco, Antonio Conti*
Sepsi, ICA e l'implementazione di una ceppoteca *Assunta Sartor*
Tubercolosi: ieri, oggi e domani *Assunta Sartor*
La diagnosi microbiologica di Sepsi e ICA integrata "One Health" *Alberto Colombo*
ICA di Genere *Paola Sabatini*
La Sepsi e le ICA: il punto di vista del Patologo Clinico *Paolo Doretto*
Data Bases Relazionali e SQL Le query dedicate Premal *Alessandro Orro*
"Discussione sulla sentenza della Corte di Cassazione III Civile n. 6386 del 3.3.2023" *Giovanni Casiraghi*
Reti collaborative microbiologiche: i POCT e gli obblighi legali (malattie sottoposte a denunce) *Giovanni Casiraghi*
Poster Marco Toni NewMicro2024 - ECMU e IVU *Graziella Bonetti*



Infezioni delle Vie Urinarie

Fabio Manoni, Barbara Pieretti

Gruppo **I**nterdisciplinare laboratorio
e clinica **A**pparato **U**rinario

GIAU

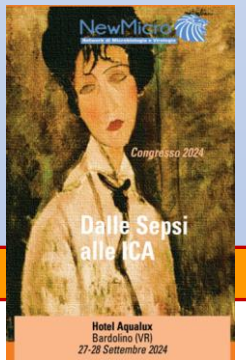
Urinary tract infections: epidemiology, mechanisms of infection and treatment options

Ana L. Flores-Mireles*, Jennifer N. Walker*, Michael Caparon, and Scott J. Hultgren

Nat Rev Microbiol. 2015

"Urinary tract infections (UTIs) are a severe public health problem and are caused by a range of pathogens, but most commonly by Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus.

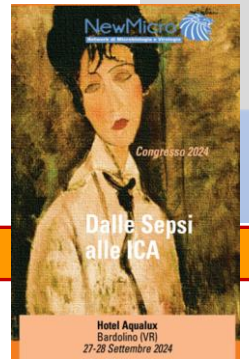
High recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly increase the economic burden of these infections".



A Pervasive and Persistent Problem....

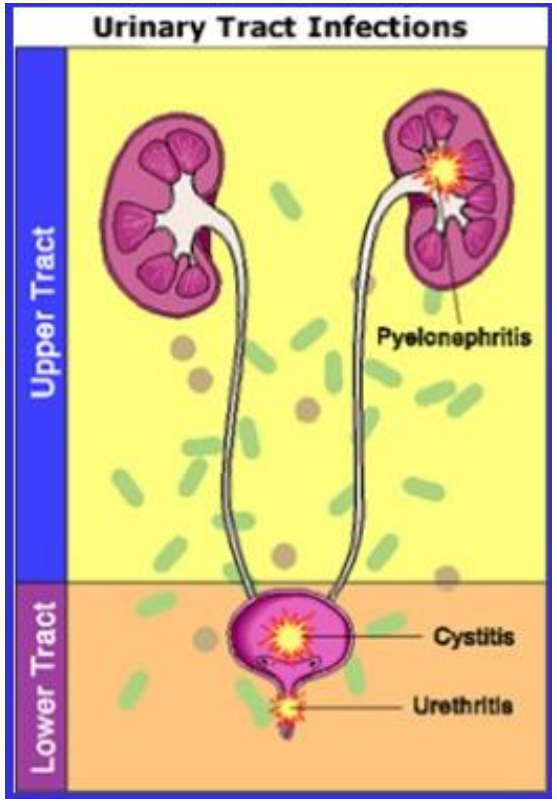
- **Le infezioni del tratto urinario (UTI) sono tra le infezioni batteriche più comuni**
- Colpiscono ogni anno **150 milioni di persone in tutto il mondo**.
- Le infezioni del tratto urinario sono tradizionalmente considerate **una malattia delle donne**, tra le quali il **50%** ne sarà colpito nel corso della loro vita. Il **25%** delle donne che presentano un primo episodio di cistite batterica va incontro a **IVU ricorrente entro 6 mesi**, alcune con più infezioni nell'anno successivo all'episodio iniziale
- Le attuali terapie non sono ottimali, poiché la prevalenza di uropatogeni multiresistenti è in aumento e il trattamento antibiotico per l'infezione acuta non preclude le recidive. Queste infezioni recidivanti possono diventare un problema di salute significativo e diminuire la qualità della vita per uomini e donne colpiti

Urinary Tract Infection: Pathogenesis and Outlook
Lisa K. McLellanand, David A. Hunstad Trends Mol Med. 2016



Infezioni delle Vie Urinarie

Le infezioni delle vie urinarie (Urinary tract infections o UTIs) sono un processo infiammatorio di natura infettiva a carico del tratto urinario o di parte di esso, caratterizzato dalla presenza di segni e sintomi delle vie urinarie associati a isolamento di microrganismi patogeni nelle urine.

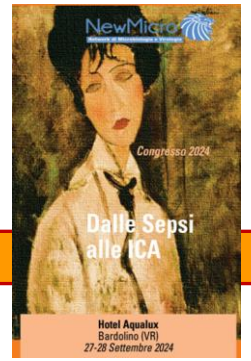


UTI non complicate

Occorrono in un apparato urinario morfologicamente e funzionalmente indenne ed in assenza di specifiche comorbidità. Le infezioni acute non complicate comprendono le infezioni delle basse vie urinarie (cistiti, uretriti, prostatiti) e delle vie urinarie superiori (pielonefriti, cistopieliti).

UTI complicate

Si manifestano in soggetti a particolare rischio di complicanze come: bambini, donne in gravidanza, immunodepressi, diabetici, pazienti portatori di catetere, pazienti con alterazioni anatomiche o funzionali dell'apparato urinario, insufficienza renale, uropatia ostruttiva, vescica neurologica.



La maggior parte delle infezioni correlate all'assistenza riguarda il tratto urinario (da sole rappresentano infatti il 35-45% del totale), cui seguono quelle del sito chirurgico, dell'apparato respiratorio e le infezioni sistemiche come sepsi e batteriemie

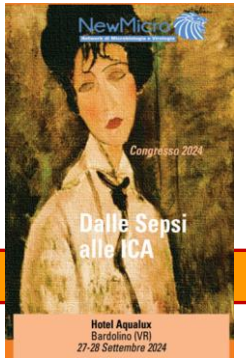
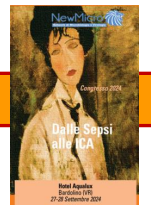


Table 1-1. Predisposing Risk Factors for UTI

Patient Population	Risk Factors
Premenopausal women of any age	• Diabetes • Diaphragm use, especially those with spermicide • History of UTI or UTI during childhood • Mother or female relatives with history of UTIs • Sexual intercourse
Postmenopausal and older adult women	• Estrogen deficiency • Functional or mental impairment • History of UTI before menopause • Urinary catheterization • Urinary incontinence
Men and women with structural abnormalities	• *Extrarenal obstruction* associated with congenital anomalies of the ureter or urethra, calculi, extrinsic ureteral compression, or benign prostate hypertrophy • *Intrarenal obstruction* associated with nephrocalcinosis, uric acid nephropathy, polycystic kidney disease, hypokalemic or analgesic nephropathy, renal lesions from sickle cell disease

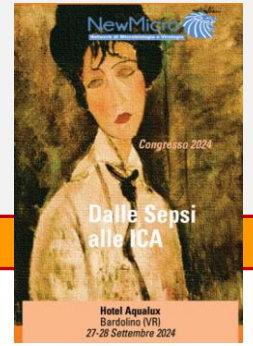
UTI = urinary tract infection.

Information from: Grabe M, Bartoletti R, Bjerklund Johansen TE, et al, for the European Association of Urology. Guidelines on Urological Infections. 2015; and Sobel JD, Kaye D. Urinary tract infections. In: Mandell GL, Bennett JE, eds. Principles and Practice of Infectious Diseases, 8th ed. Philadelphia: Elsevier Saunders, 2014:886-913.

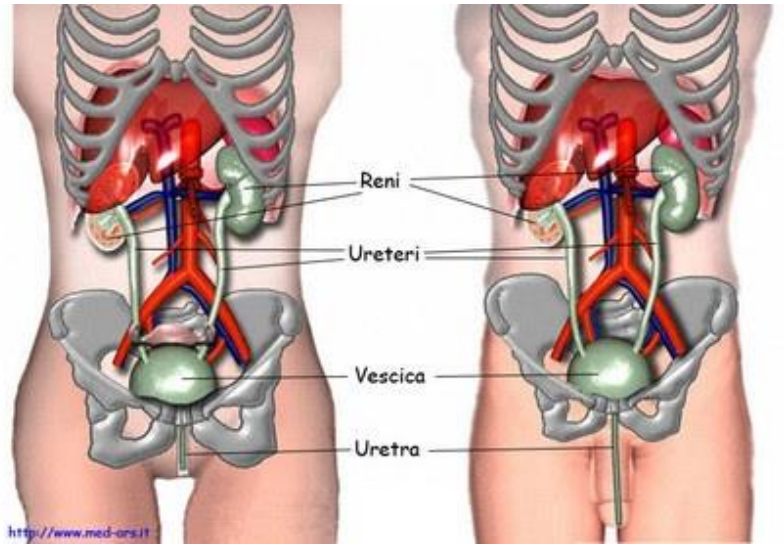


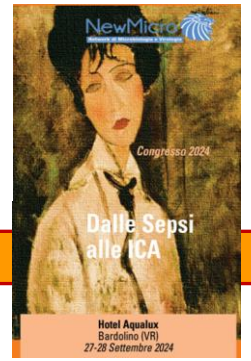
Le categorie più a **rischio di infezioni delle vie urinarie** sono rappresentate da:

- donne **incinte e anziani**
- persone affette da **diabete** (tipo 1 o tipo 2)
- persone **immunodepresse** (a causa di infezioni o malattie da HIV o in trattamento con farmaci immunosoppressori o chemioterapici)
- persone con **calcoli** delle vie urinarie o dei reni
- persone con **ostruzione dell'uretra** (stenosi uretrale, ipertrofia prostatica, prolasso dell'utero o della vescica)
- persone con **vescica neurologica**
- portatori di **catetere vescicale**, in particolare nel corso di un ricovero ospedaliero (le infezioni delle vie urinarie rappresentano il 35-40% di tutte le infezioni ospedaliere).



Anatomia vs sesso-età-gravidanza





TERMINOLOGIA

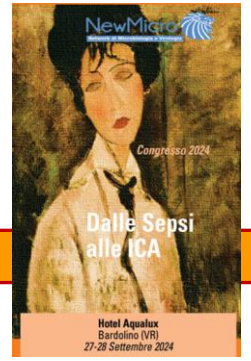
Urinary Tract Infection**: the combination of a pathogen(s) within the urinary system and symptoms and/or inflammatory response to the pathogen(s) requiring treatment

Bacteriuria*: presence of bacteria in urine revealed by quantitative culture or microscopy.

Asymptomatic bacteriuria*: presence of bacteriuria in urine revealed by quantitative culture or microscopy in a sample taken from a patient without any typical symptoms of lower or upper urinary tract infection. In contrast with symptomatic bacteriuria, the presence of asymptomatic bacteriuria should be confirmed by two consecutive urine samples.

Significant bacteriuria*: for laboratory purposes the widely applied definition in the UK is 10^4 cfu/ml.
For some specific patient groups there is evidence for lower thresholds:
- women with symptomatic UTI $\geq 10^2$ cfu/ml,
- men $\geq 10^3$ cfu/ml (if 80% of the growth is due to a single organism).

**SIGN 88 • Management of suspected bacterial urinary tract infection in adults (2012)*
***American Urological association • Adult Urinary tract infection (2016)*
****European Association of Urology • Guidelines on Urological Infection (2013)*



TERMINOLOGIA

Symptomatic bacteriuria*: presence of bacteriuria in urine revealed by quantitative culture or microscopy in a sample taken from a patient, or the typical symptoms of lower or upper urinary tract infection. The presence of symptomatic bacteriuria can be established with a single urine sample.

Contamination**: organisms are introduced during collection or processing of urine. No health care concerns.

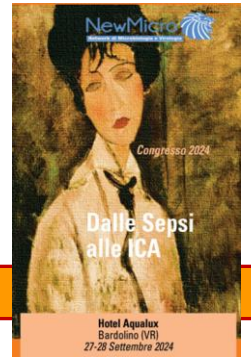
Colonization**: organisms are present in the urine, but are causing no illness or symptoms (asymptomatic bacteriuria). Depending on the circumstances, significance is variable, and the patient often does not require treatment.

Recurrent UTI**:** occurs after documented infection that had resolved

Reinfection UTI**:** a new event with reintroduction of bacteria into urinary tract

Persistent UTI**:** UTI caused by same bacteria from focus of infection

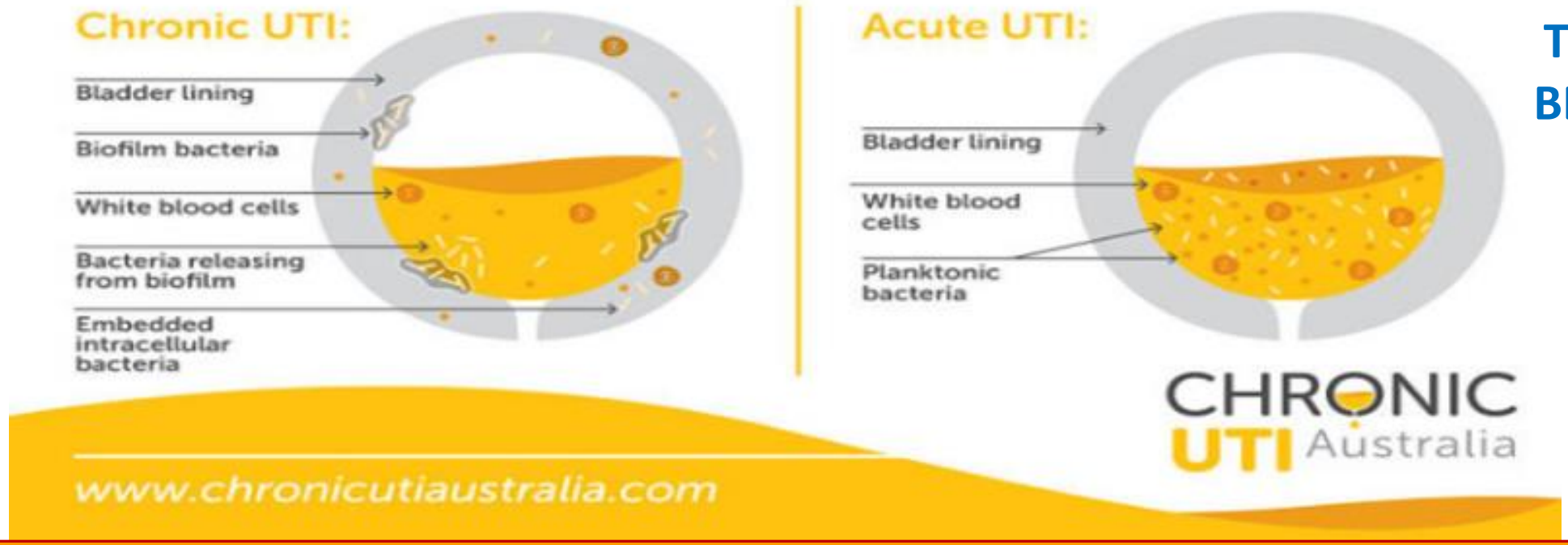
**SIGN 88 • Management of suspected bacterial urinary tract infection in adults (2012)*
***American Urological association • Adult Urinary tract infection (2016)*



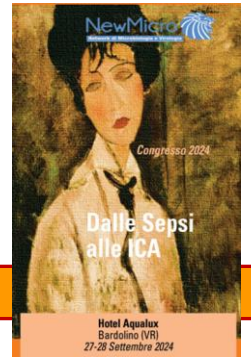
A **recurrent urinary tract infection** is officially defined as three episodes of a UTI in the previous 12 months or two episodes within the previous 6 months.

Reinfection refers to an infection where the pathogen is eradicated by treatment, then the same or a different pathogen ascends the urinary tract to cause a new infection.

Persistence means the pathogen that caused the UTI is not completely cleared from the bladder by treatment, remains detectable in the urine, and after treatment returns to a level that once again causes symptoms of infection. This cycle of persistence can repeat indefinitely, feeling like a new infection each time. A persistent infection is also called a **chronic urinary tract infection**.



THE DIFFERENCE BETWEEN ACUTE UTI AND CHRONIC UTI



Urinary tract infections: epidemiology, mechanisms of infection and treatment options

Flores-Mireles et al. *Nat Rev Microbiol*. 2015 May ; 13(5): 269–284

Epidemiologia

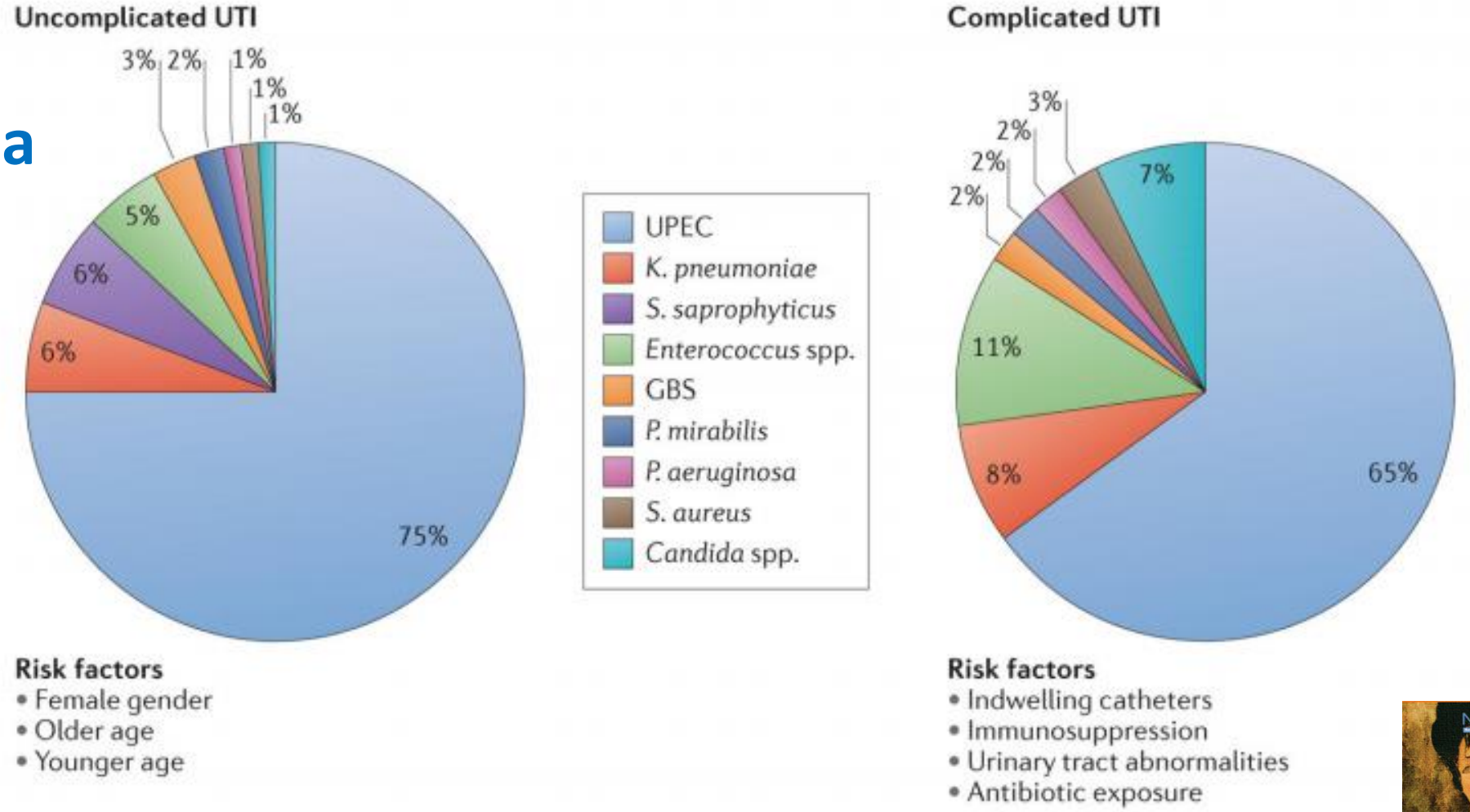
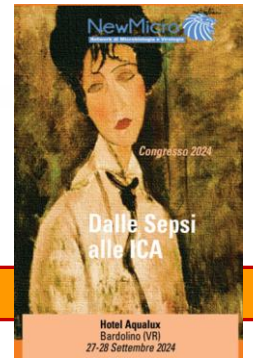


Figure 1. Epidemiology of urinary tract infections



Urinary Tract Infections

PSAP 2018 BOOK 1 • *Infectious Diseases*

Table 1-2. Uropathogens by Type of UTIs

Type	Common Uropathogens
Uncomplicated UTI	*E. coli* *S. saprophyticus* *Enterococcus* spp. *K. pneumoniae* *P. mirabilis*
Complicated UTI	Similar to uncomplicated UTI Antibiotic-resistant *E. coli* *P. aeruginosa* *Acinetobacter baumannii* *Enterococcus* spp. *Staphylococcus* spp.
CA-UTI	*P. mirabilis* *Morganella morganii* *Providencia stuartii* *C. urealyticum* *Candida* spp.
Recurrent UTI	*P. mirabilis* *K. pneumoniae* *Enterobacter* spp. Antibiotic-resistant *E. coli* *Enterococcus* spp. *Staphylococcus* spp.

CA-UTI = catheter-associated urinary tract infection; UTI = urinary tract infection.

Information from: Sobel JD, Kaye D. Urinary tract infections. In: Mandell GL, Bennett JE, eds. Principles and Practice of Infectious Diseases, 8th ed. Philadelphia: Elsevier Saunders, 2014:886-913.

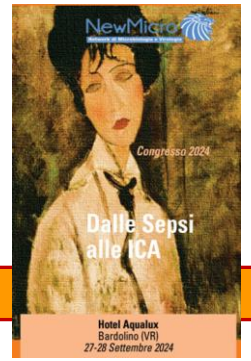
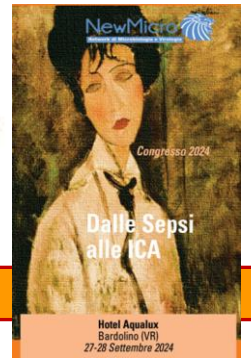


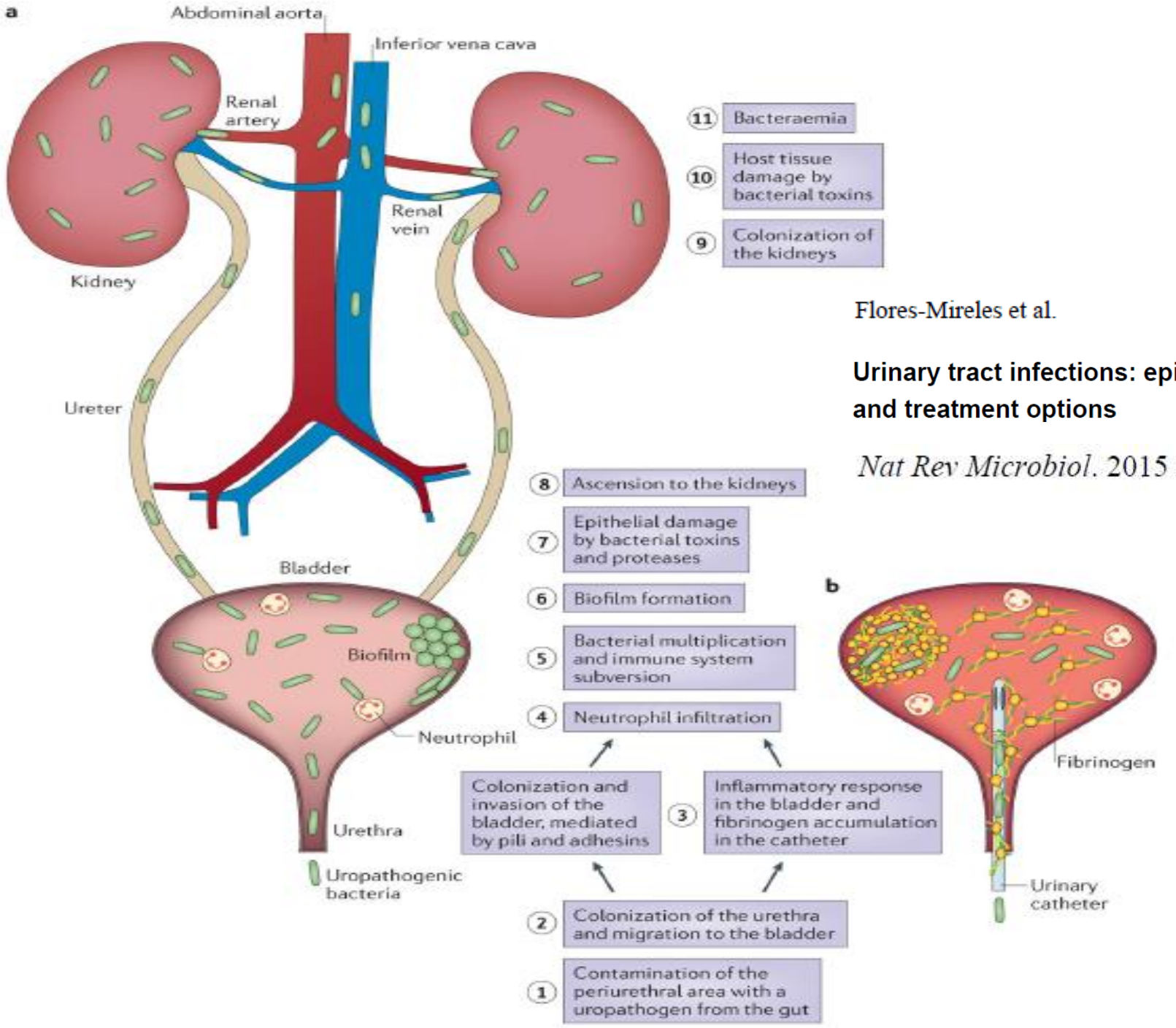
Table 1-3. Definition of Types of UTIs

Category	Definition
Uncomplicated UTI	• Lower urinary symptoms (dysuria, frequency, and urgency) in otherwise healthy non-pregnant women
Complicated UTI	• Pregnant women, men, obstruction, immunosuppression, renal failure, renal transplantation, urinary retention from neurologic disease, and individuals with risk factors that predispose to persistent or relapsing infection (e.g., calculi, indwelling catheters or other drainage devices) • Health care associated
CA-UTI	• Presence of indwelling urinary catheters with signs and symptoms of UTI and no other source of infection • Presence of ≥ 10^3 CFU/mL in a single catheter urine specimen or in a midstream urine, despite removal of urinary catheter in the previous 48 hr
Asymptomatic bacteriuria	• *Women*: Two consecutive voided urine specimens with isolation of the same bacteria at ≥ 10^5 CFU/mL • *Men*: A single, clean-catch, voided urine specimen with 1 bacteria isolated 10^5 CFU/mL • A single catheterized urine specimen with 1 bacteria isolated ≥ 10^2 CFU/mL

CA-UTI = catheter-associated UTI.

Information from: Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;5:e103-20; Nicolle LE, Bradley S, Colgan R, et al. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis 2005;5:643-54; Hooton TM, Bradley SF, Cardenas DD, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin Infect Dis 2010;5:625-63.

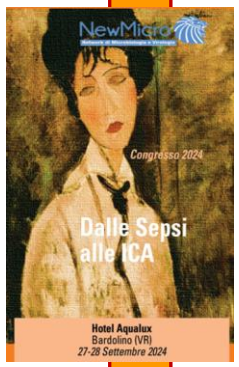




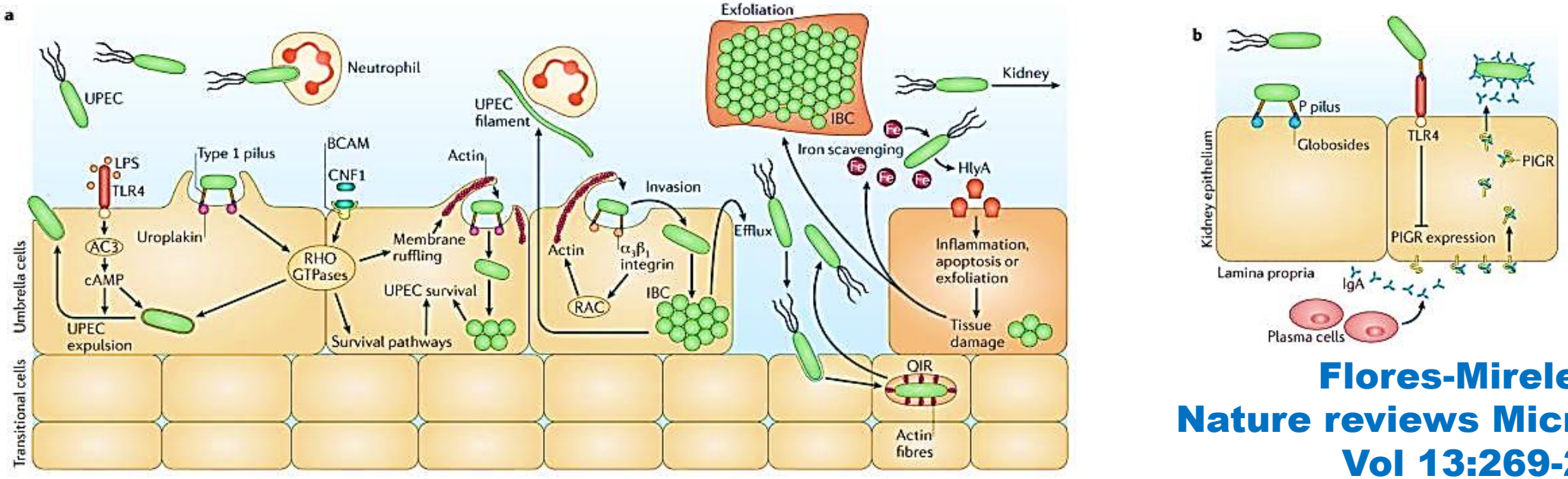
Flores-Mireles et al.

Urinary tract infections: epidemiology, mechanisms of infection and treatment options

Nat Rev Microbiol. 2015 May ; 13(5): 269–284



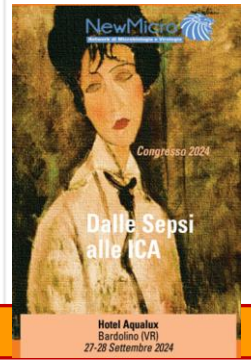
Virulence factors of uropathogenic *E.coli* that contribute to urinary tract infections



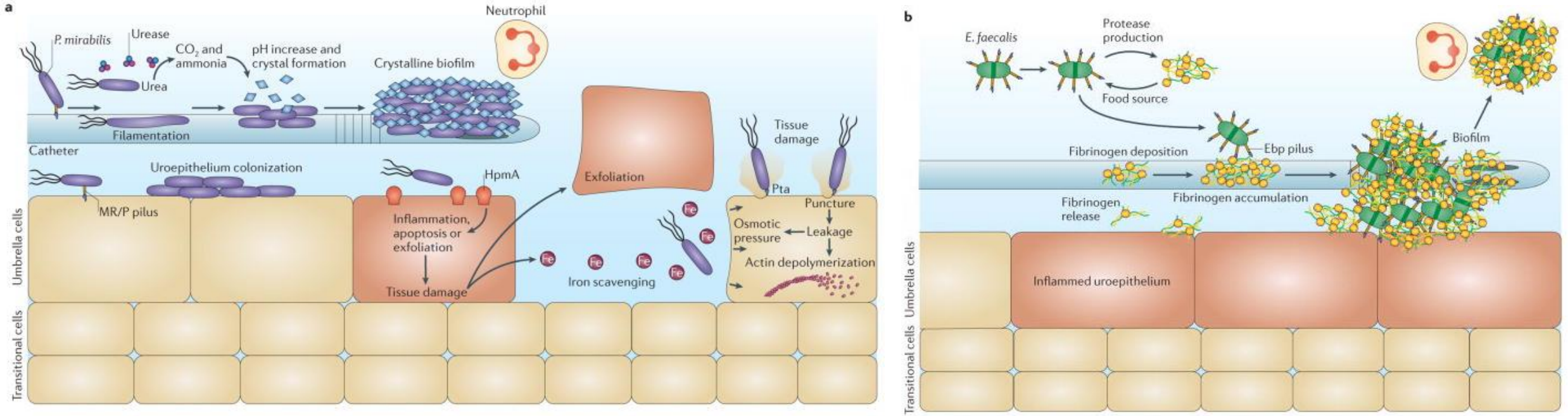
Flores-Mirele AL et al, Nature reviews Microbiology, Vol 13:269-284, 2017

a | In the bladder, uropathogenic *Escherichia coli* (UPEC) expression of type 1 pili is essential for colonization, invasion and persistence. The type 1 pilus adhesin, FimH, binds mannosylated uroplakins and integrins that coat the surface of umbrella cells. Uroplakin binding by FimH induces actin rearrangement and bacterial internalization via unknown mechanisms. FimH–$\alpha_3\beta_1$ integrin interactions induce actin rearrangement via activation of RHO-family GTPases (such as RAC proteins), resulting in bacterial invasion. Inside the host cell, UPEC can subvert host defences and resist antibiotic treatment. However, lipopolysaccharide (LPS) released by UPEC is sensed by Toll-like receptor 4 (TLR4), which induces cyclic AMP (cAMP) production via adenylyl cyclase 3 (AC3) activation, resulting in exocytosis of vesicular UPEC across the apical plasma membrane. UPEC subverts this innate defence mechanism by escaping into the cytoplasm, where it then multiplies to form intracellular bacterial communities (IBCs). Maturation of IBCs causes bacterial dispersal and allows the invasion of other host cells, which enables UPEC to re-enter the IBC cycle. Alternatively, UPEC can establish quiescent intracellular reservoirs (QIRs) in the underlying transitional cells. QIRs consist of 4–10 non-replicating bacteria within membrane-bound compartments encased in F-actin and can remain viable for months. In addition, UPEC survives within the harsh bladder environment by secreting several factors that are important for nutrient acquisition. The toxin α-haemolysin (HlyA) promotes host cell lysis through pore formation, facilitating iron release and nutrient acquisition. The siderophores expressed by UPEC allow the bacterium to scavenge iron and thus promote survival during a urinary tract infection (UTI). HlyA also triggers epithelial exfoliation to promote the spread of UPEC to other hosts following urine expulsion or to expose deeper layers of the uroepithelium for QIRs. Cytotoxic necrotizing factor 1 (CNF1) is also important for host cell remodelling and functions by binding to the receptor basal cell adhesion molecule (BCAM) on host cells to induce constitutive activation of the RHO GTPases RAC1, RHOA and cell division control 42 (CDC42), resulting in actin cytoskeletal rearrangements and membrane ruffling. Activation of RAC1 also induces the host cell anti-apoptotic and pro-survival pathways, preventing apoptosis of colonized epithelial cells and allowing the UPEC population to expand. The extracellular survival of UPEC also requires evasion of the innate immune system by the adoption of a filamentous morphology, which renders the bacterium more resistant to neutrophil killing than their bacillary form. **b** | UPEC colonization of the kidneys is dependent on expression of pyelonephritis-associated (P) pili, which bind globoside-containing glycolipids lining the renal tissue. The P pilus adhesin, PapG, also interacts with TLR4, reducing the expression of polymeric immunoglobulin receptor (PIGR). This results in impaired immunoglobulin A (IgA) transport across the epithelium, thereby modulating the local secretory antibody immune response and preventing UPEC opsonization and clearance.

Manoni F, Pieretti B – Newmicro 2024

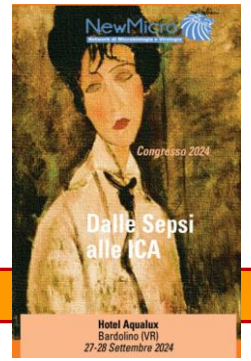


Mechanisms of pathogenesis during catheter-associated urinary tract infections



a | Catheter-associated urinary tract infections (CAUTIs) mediated by *Proteus mirabilis* depend on the expression of mannose-resistant *Proteus*-like (MR/P) pili for initial attachment, and for biofilm formation on the catheter and in the bladder. Subsequent urease production induces the formation of calcium crystals and magnesium ammonium phosphate precipitates in the urine through the hydrolysis of urea to carbon dioxide and ammonia, resulting in a high pH. The production of extracellular polymeric substances by bacteria attached to the catheter traps these crystals, allowing the formation of a crystalline biofilm, which protects the community from the host immune system and from antibiotics. In addition, these structures prevent proper urine drainage, resulting in reflux and promoting the progression to pyelonephritis, septicaemia and shock. Finally, production of the bacterial toxins haemolysin (HpmA) and *Proteus* toxic agglutinin (Pta) is important for tissue destruction and bacterial dissemination to the kidneys. HpmA induces pore formation by inserting itself into the cell membrane and destabilizing the host cell, causing tissue damage, exfoliation and nutrient release. Pta punctures the host cell membrane, causing cytosol leakage and resulting in osmotic stress and depolymerization of actin filaments, thus compromising the structural integrity of the cell. The release of nutrients via these toxins also allows the bacteria to scavenge iron using siderophores. **b** | *Enterococcus faecalis* pathogenesis during CAUTIs depends on catheter implantation, which results in bladder inflammation and causes fibrinogen release, deposition onto the catheter, and accumulation. *E. faecalis* takes advantage of the presence of fibrinogen and uses it as a food source through the production of proteases. *E. faecalis* also binds fibrinogen through the endocarditis- and biofilm-associated (Ebp) pilus, allowing the formation of biofilms that protect the bacteria against the immune system.

Flores-Mirele AL et al, Nature reviews Microbiology, Vol 13:269-284, 2017



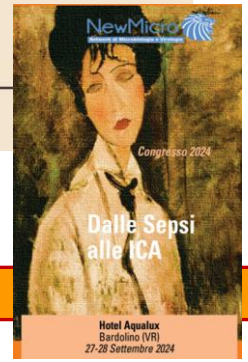
"Catheter replacement in catheter-associated urinary tract infection: current state of evidence"

European Journal of Clinical Microbiology & Infectious Diseases (2024) 43:1631–1637
https://doi.org/10.1007/s10096-024-04878-9

Table 1 Definition of catheter associated urinary tract infection in the 4 included studies

	Clinical	Microbiological	Pyuria
Kumazawa	Catheter for ≥ 21 days	$> 10^4$ CFU/ml	> 5 WBC/HPF
Raz	Indwelling catheter Fever or hypothermia, other signs of infection (leukocytosis or leukopenia)	$> 10^3$ (one organism) or 10^4 (two organisms) CFU/ml	
Darouiche	Indwelling catheter ≥ 1 symptom: fever, suprapubic or flank discomfort, bladder spasm, increased spasticity, worsening dysreflexia and cloudy urine	$> 10^5$ CFU/ml	> 10 WBC/HPF
Babich	Catheter for ≥ 7 days Systemic inflammatory response syndrome (SIRS) Clinical exam and chest imaging to exclude other causes of infection	$> 10^3$ (one organism) or 10^4 (two organisms) CFU/ml or bacteremia caused by an uropathogen Bacteremia caused by an uropathogen	> 10 WBC/ml

Legend WBC/HPF: white blood cells/high-power field. CFU: colony forming units



Precision antimicrobial therapeutics: the path of least resistance?

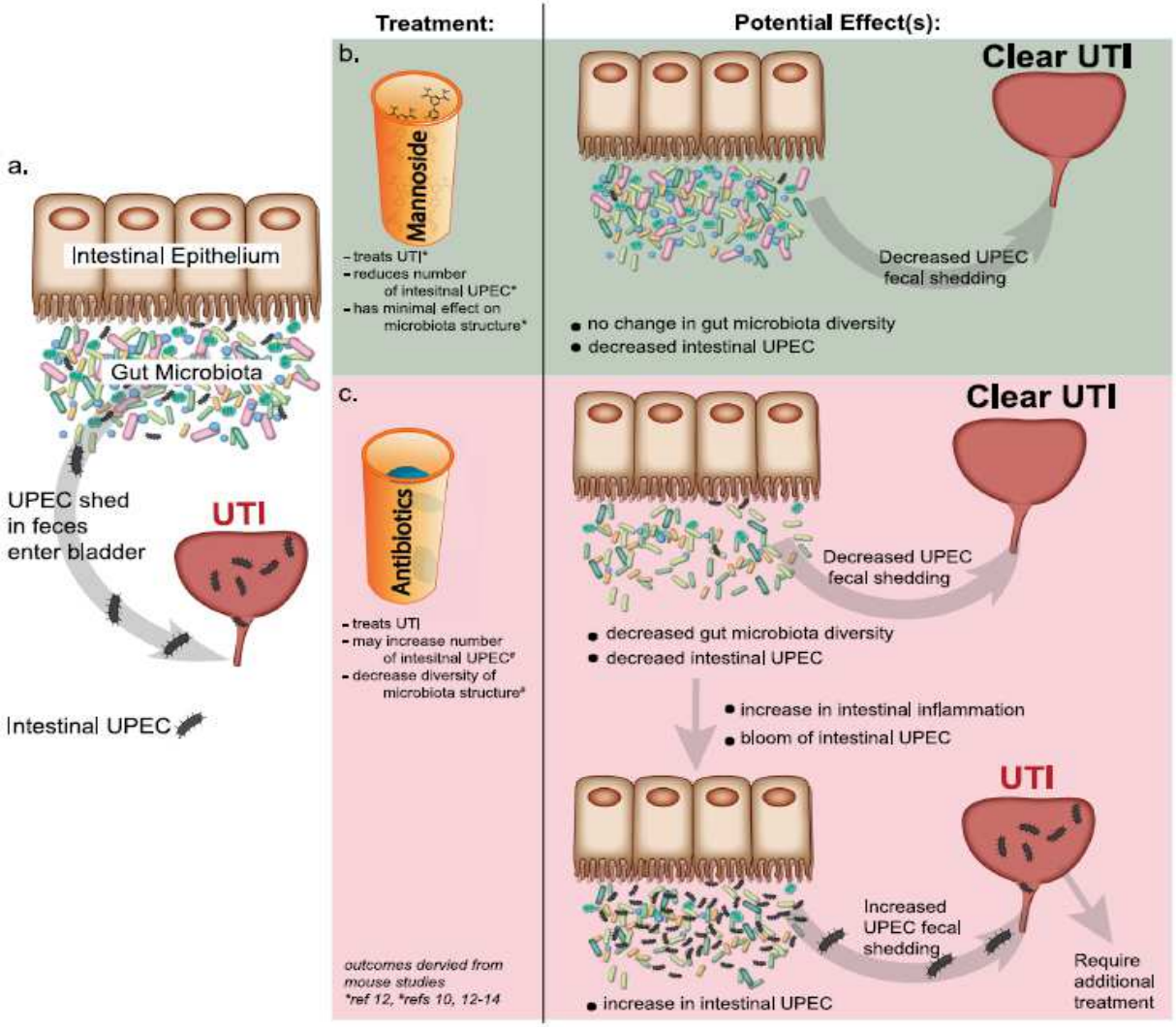


Fig. 1 Potential effects of oral mannoside and antibiotic treatment on the intestinal UPEC population. **a** Intestinal UPEC reach the bladder and can cause UTI after being shed in the feces. **b** Oral mannoside treatment targets and reduces the UPEC intestinal population and simultaneously treats and clears UTI in the bladder with minimal effects on the overall structure/diversity of the gut microbiota. **c** Conversely, oral treatment with clinically relevant broad-spectrum antibiotics, like ciprofloxacin, can treat and clear UTI but reduces the overall abundance and diversity of the gut microbiota. The resulting intestinal inflammation caused by antibiotic treatment may promote intestinal *E. coli* colonization (including UPEC) and thus can lead to increase UPEC fecal shedding, promoting recurrent UTI

CN Spaulding et al.

npj Biofilms and Microbiomes (2018) 4

CONCLUSIONS

Broad-spectrum antibiotics are invaluable tools for the treatment and prevention of disease; however, the rise of antibiotic-resistant pathogens has made treating individuals with single and multidrug-resistant infections challenging. Further, the increasing number of studies finding that antibiotic-mediated disruption of the microbiota may be detrimental to the host suggests that treating individuals with antibiotics, particularly broad-spectrum antibiotics, has some negative consequences. Therefore, developing precision or "ultra-narrow" spectrum antimicrobials, like mannosides, that are designed to target a specific organism while leaving the remaining microbial community untouched is needed (Fig. 2). Developing therapies that target the host reservoir of pathogens, rather than simply the site of infection, may help to reduce disease burden and/or prevent recurrence.

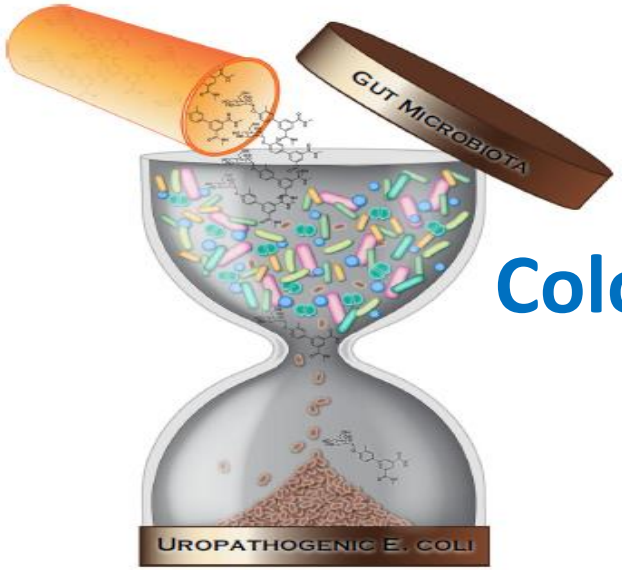
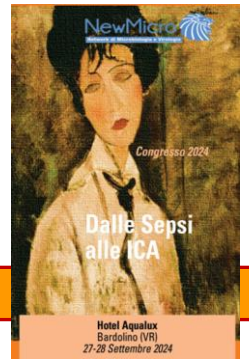


Fig. 2 Precision therapeutics target a specific organism while leaving the remainder a microbiota community untouched. Artist rendering of how mannoside treatment (compound from prescription bottle) selectively extirpates UPEC from the gut microbiota

Colonizzazione



Review

Microbial Biofilms in Urinary Tract Infections and Prostatitis: Etiology, Pathogenicity, and Combating strategies

Pathogens 2016, 5, 65

Cristina Delcaru [1], Ionela Alexandru [2,3], Paulina Podgoreanu [2,3], Mirela Grosu [3], Elisabeth Stavropoulos [1], Mariana Carmen Chifiriuc [1,3,*] and Veronica Lazar [1,3]

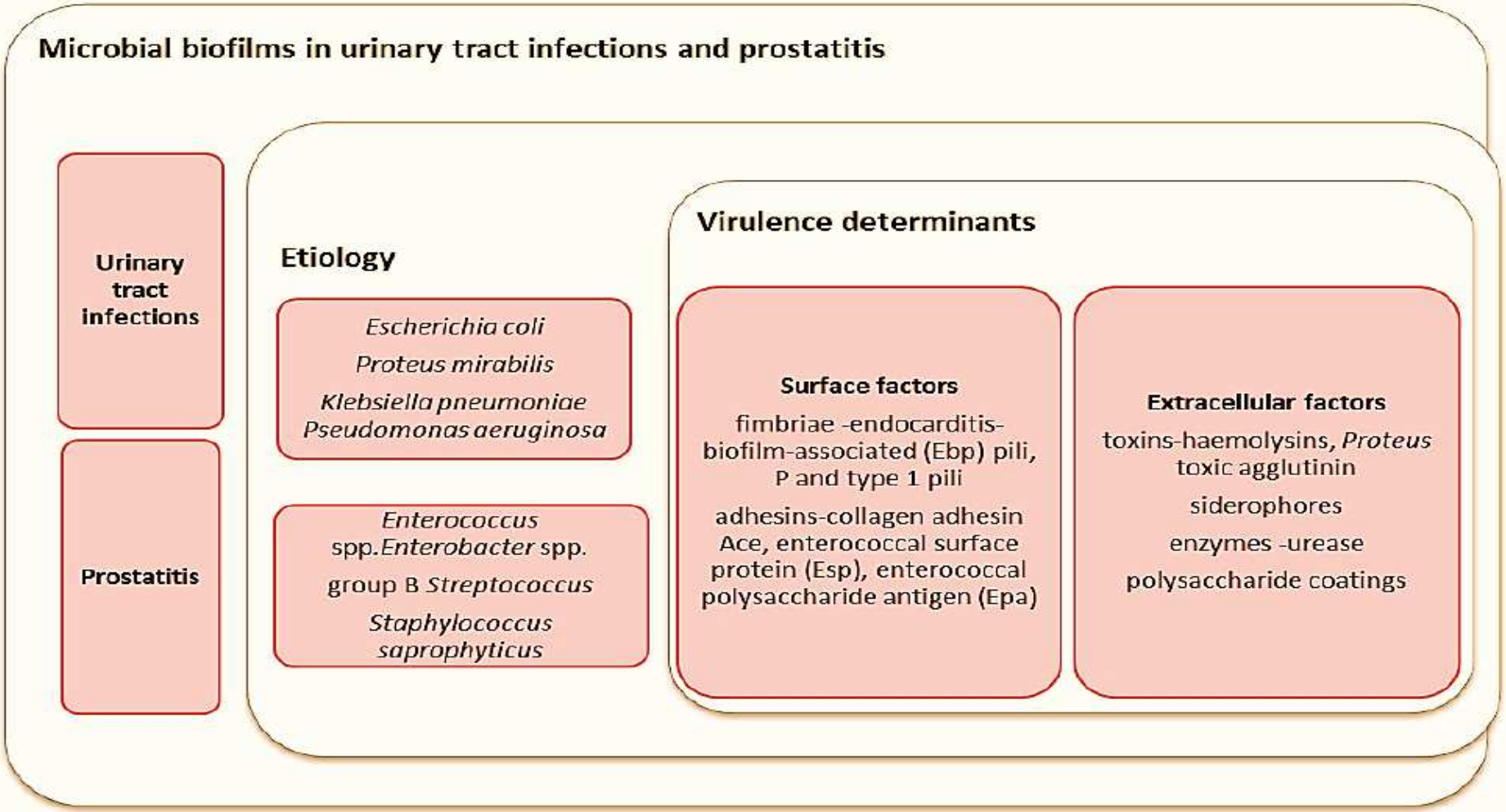
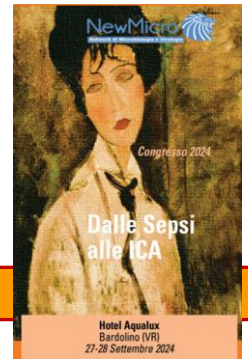


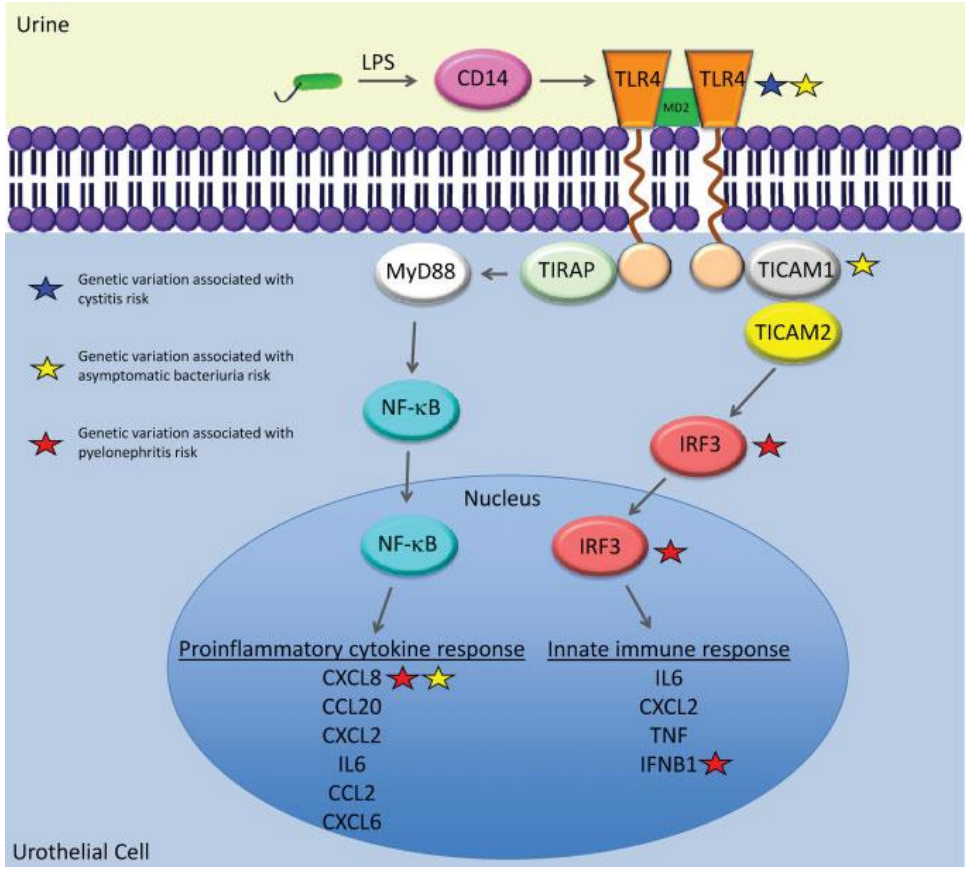
Figure 1. Etiology and virulence determinants of urinary tract microbial biofilms.

Biofilm



The Genetics of Urinary Tract Infection and the Innate Defense of the Kidney and Urinary tract

Ambite I et al, Journal of Pediatric Genetics, Vol 5, No 1: 25-32, 2016

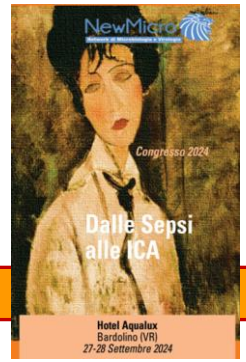


Patients with ABU can carry the same bacterial strain in their bladders for several months or years without developing symptoms of UTI.[37] Murine genetic models that interfere

Conclusion

The innate defense of the kidney and urinary tract isa comple and multifactorial process. Several components work in concert to prevent and immediattely clear invading pathogens. As we review in the manuscriptdifferent variations in the same gene can lead to significantly different phenotypes ranging from ABU to APN and renal scarring. While several significant mutations have been identified in known critical components, much is still to be discovered. Antimicrobial peptides and epithelial cell functions have not been well studied. For example, mice with intercalated cell-targeted dysfunctions have increased risk of UTI with bacterial challenge. [75,76] Well phenotyped cohorts of these conditions and genome-wide studies are needed to identify the entire milieu of the innate defense that may be targeted for novel therapeutics in preventing or treating UTIs.

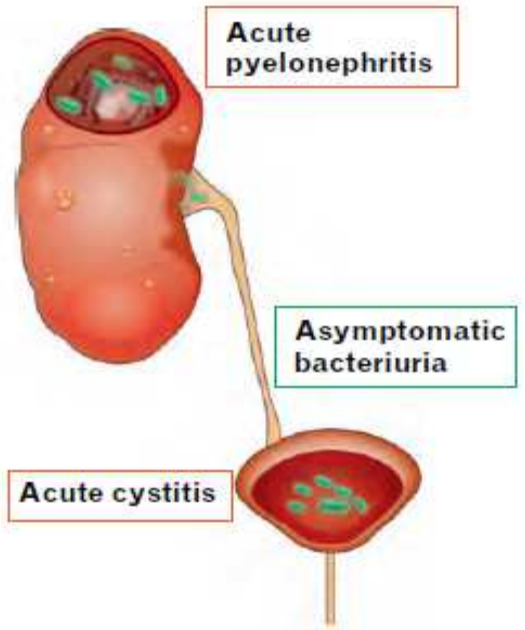
Fig. 1 Schematic of TLR4 (toll-like receptor 4) signaling: MyD88 (myeloid differentiation primary response 88)-dependent and MyD88-independent pathways mediate TLR4 signaling. Genetic variations in key proteins or inflammatory mediators within TLR4 signaling pathways can result in increased asymptomatic bacteriuria (white stars), cystitis (striped stars), or pyelonephritis (black stars) risk. Pathway crosstalk and other involved pathway proteins are present but omitted from the figure for illustration purposes. ABU, asymptomatic bacteriuria; APN, acute pyelonephritis; CCL, chemokine C-C motif ligand; CCL20, chemokine (C-C motif) ligand 20; CXCL8, chemokine (C-X-C motif) ligand 8; CXCR, C-X-C chemokine receptor; ICAM, intercellular adhesion molecule; IFNB1, interferon β 1, fibroblast; IRF, interferon regulatory factor; IRF3, interferon regulatory factor 3; LPS, lipopolysaccharide; NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; PRR, pattern recognition receptors; PTX3, pentraxin 3; TGFB1, transforming growth factor β 1; TICAM, toll-like receptor adaptor molecule 1; TIRAP, toll-IL 1 receptor (TIR) domain containing adaptor protein; TLR, toll-like receptor; TNF, tumor necrosis factor; UMOD, uromodulin; UPEC, uropathogenic *Escherichia coli*; UTIs, urinary tract infections; VEGF, vascular endothelial growth factor; VUR, vesicoureteral reflux.



Innate immunity and genetic determinants of urinary tract infection susceptibility

Godaly G et al, Curr Opin Infect Dis, Vol 28:88–96, 2015

(a)



(b)

APN, sepsis or renal scarring

Genotype	Ref	Genotype	Ref
mCxcr2$^{-/-}$	[33, 36, 37]	*Camp*$^{-/-}$	[39]
Irf3$^{-/-}$	[11]	*Thp*$^{-/-}$	[45]
Ifnb1$^{-/-}$	[11]	*Tlr11*$^{-/-}$	[41]

Asymptomatic bacteriuria

Genotype	Ref	Genotype	Ref
C3H/HeJ *Tlr4* mutant	[23, 26, 28, 30, 33]	*Tram*$^{-/-}$	[30]
Tlr4$^{-/-}$	[30]	*Myd88*$^{-/-}$	[30]
Trif$^{-/-}$	[30]		

*Bladder infection**

Genotype	Ref	Genotype	Ref
Car2$^{-/-}$	[42]	*Tlr5*$^{-}$	[47]
Cox2$^{-/-}$	[46]		

(c)

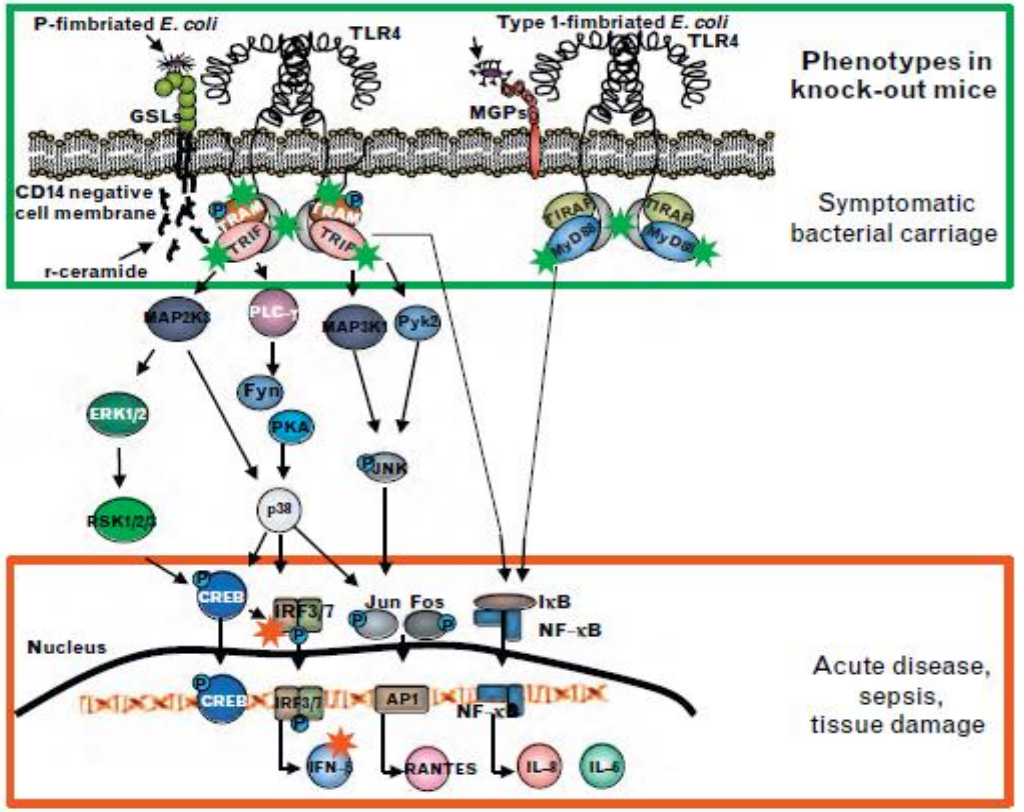
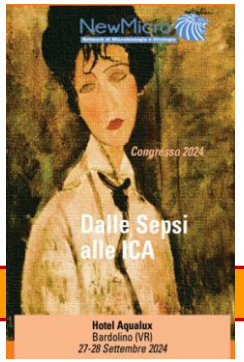


FIGURE 1. Genetic determinants of urinary tract infection susceptibility in the murine and human urinary tract infection model. (a) Infections of the urinary tract give rise to acute pyelonephritis, acute cystitis or asymptomatic bacteriuria. (b) Genetic control at the proximal level of the TLR4 signalling cascade (green) determines whether an innate immune response will be activated by infection. Gene deletions (*Tlr4, MyD88, Trif, Tram*) abrogate response, as well as symptoms and disease, thus protecting the infected host from acute disease and tissue damage. Genetic control at the distal level of the TLR4 signalling cascade (red) determines the efficiency of the antibacterial defense. Gene deletions (*Irf3, Ifnb1*) cause acute pyelonephritis and renal tissue damage. In addition, perturbations of neutrophil recruitment and function drastically increase the susceptibility to APN and renal scarring (*mCxcr2*). (c) Genes associated with UTI susceptibility in the murine UTI model. *IL-1 and inflammasome genes [48].

CONCLUSION

UTI susceptibility is influenced by the genetic make up of the host, especially by genes that regulate the innate immune response to infection. Functionally relevant genes are regulatory rather than structural, suggesting that control of gene expression is essential. Thus, unlike the rare, monogenetic disorders defined by gene loss, UTI susceptibility is defined by the efficiency of the host defense. Furthermore, emerging data suggest that different molecular response pathways and genes characterize patients with APN, acute cystitis or ABU. To further validate the power of genetic variants in UTI risk assessment, clinical study criteria should be coordinated between study centres.



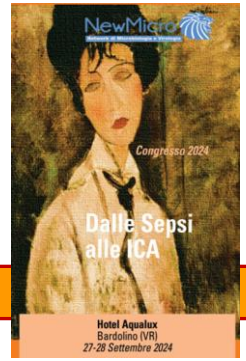
The Vaginal Microbiota and urinary Tract Infection

Stapleton AE, microbiol Spectrum, Vol 4, No 6: UTI-0025, 2016

ABSTRACT The vagina is a key anatomical site in the pathogenesis of urinary tract infection (UTI) in women, serving as a potential reservoir for infecting bacteria and a site at which interventions may decrease the risk of UTI.

The vaginal microbiota is a dynamic and often critical factor in this pathogenic interplay, because changes in the characteristics of the vaginal microbiota resulting in the loss of normally protective Lactobacillus spp. increase the risk of UTI.

These alterations may result from the influence of estrogen deficiency, antimicrobial therapy, contraceptives, or other causes. Interventions to reduce adverse effects on the vaginal microbiota and/or to restore protective lactobacilli may reduce the risks of UTI.



Does the Urinary Microbiome Play a Role in Urgency Urinary Incontinence and Its Severity?

Karstens et al, Cellular and Infection Microbiology, Vol 6, No 78, 2016

TABLE 2 | Mean relative abundance of bacteria (as percent) in UUI and control urine; reported by Phyla: Family.

	Case (%)	Control (%)	*p*-values
Firmicutes	48.7	42.7	0.60
Bacillaceae	20.3	17.4	0.57
Lactobacillaceae	19.6	19.0	0.98
Lachnospiraceae	2.4	< 2.0	0.69
Proteobacteria	25.0	15.9	0.16
Enterobacteriaceae	6.9	2.5	0.26
Comamonadaceae	5.9	2.3	0.08
Actinobacteria	11.6	19.3	0.40
Micrococcaceae	5.9	7.0	0.60
Bifidobacteriaceae	< 2.0	9.4	0.51
Bacteroidetes	8.4	17.6	0.30
Prevotellaceae	3.1	14.2	0.20
Flavobacteriaceae	2.2	< 2.0	0.35

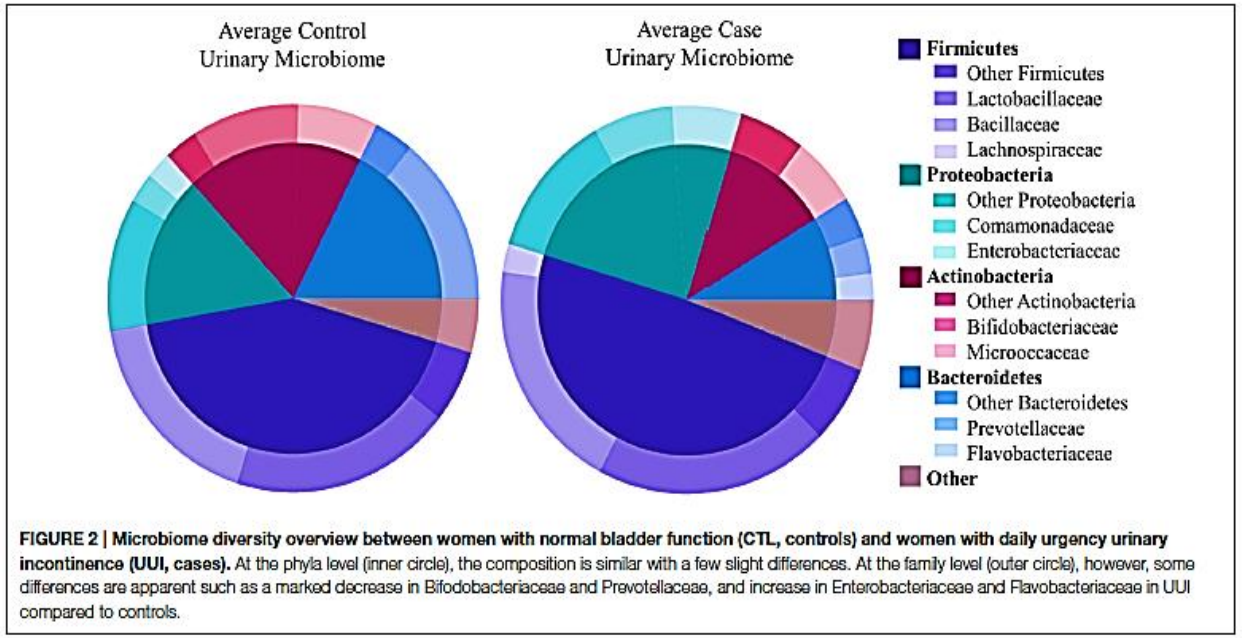


FIGURE 2 | Microbiome diversity overview between women with normal bladder function (CTL, controls) and women with daily urgency urinary incontinence (UUI, cases). At the phyla level (inner circle), the composition is similar with a few slight differences. At the family level (outer circle), however, some differences are apparent such as a marked decrease in Bifodobacteriaceae and Prevotellaceae, and increase in Enterobacteriaceae and Flavobacteriaceae in UUI compared to controls.

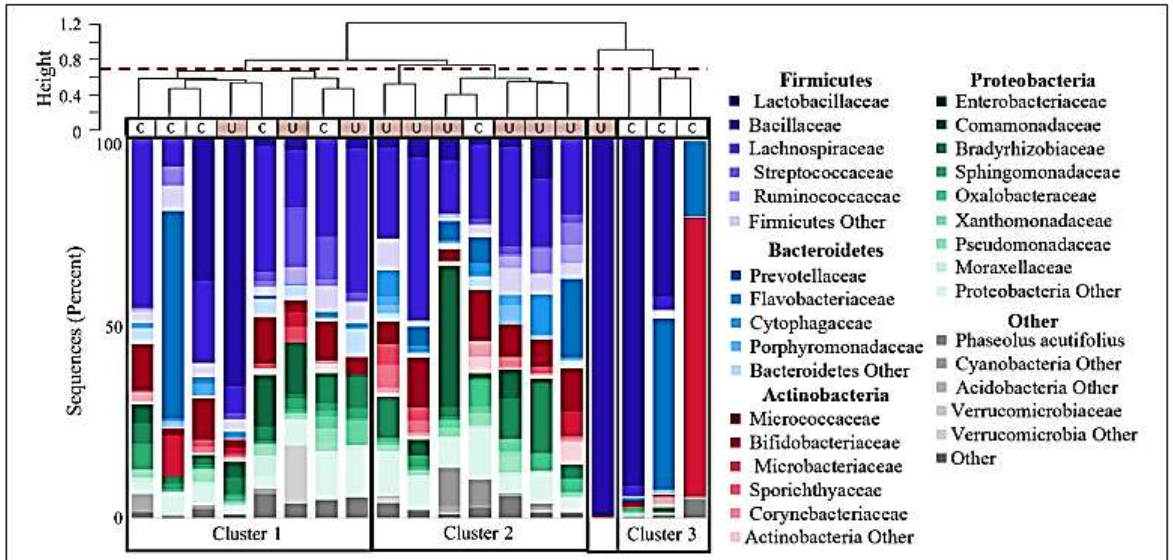
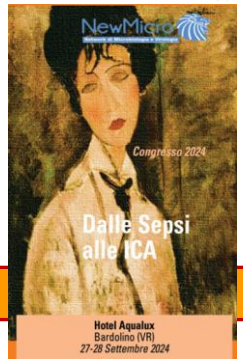


FIGURE 5 | Clustering of participants based on urinary microbiome profiles. The dendrogram (upper) is based on hierarchical clustering of the weighted Unifrac distances between the bacteria found in urine samples from controls (C) and women with UUI (U). The dashed line indicates where the clades were divided into three clusters: Cluster 1 which is comprised of five controls and three cases; Cluster 2 which is predominantly cases (six cases, one control), and Cluster 3 which is only controls. The sample type is indicated by the rectangle below the cluster bar: U, case; C, control. One case sample did not fall into a cluster at this level. The stacked bar plots below the heatmap represent the relative abundance of the bacterial families identified by 16S sequences present in each urine specimen. Bacterial families with an overall mean abundance <0.5%, are grouped as "Other." The main color is indicative of the bacterial phyla with the shade indicative of bacteria family.



The Female Urinary Microbiome: a Comparison of Women with and without Urgency Urinary Incontinence

Pearce et al, Am J Obstet Gynecol, 213(3): 347.e1–347.e11, 2015

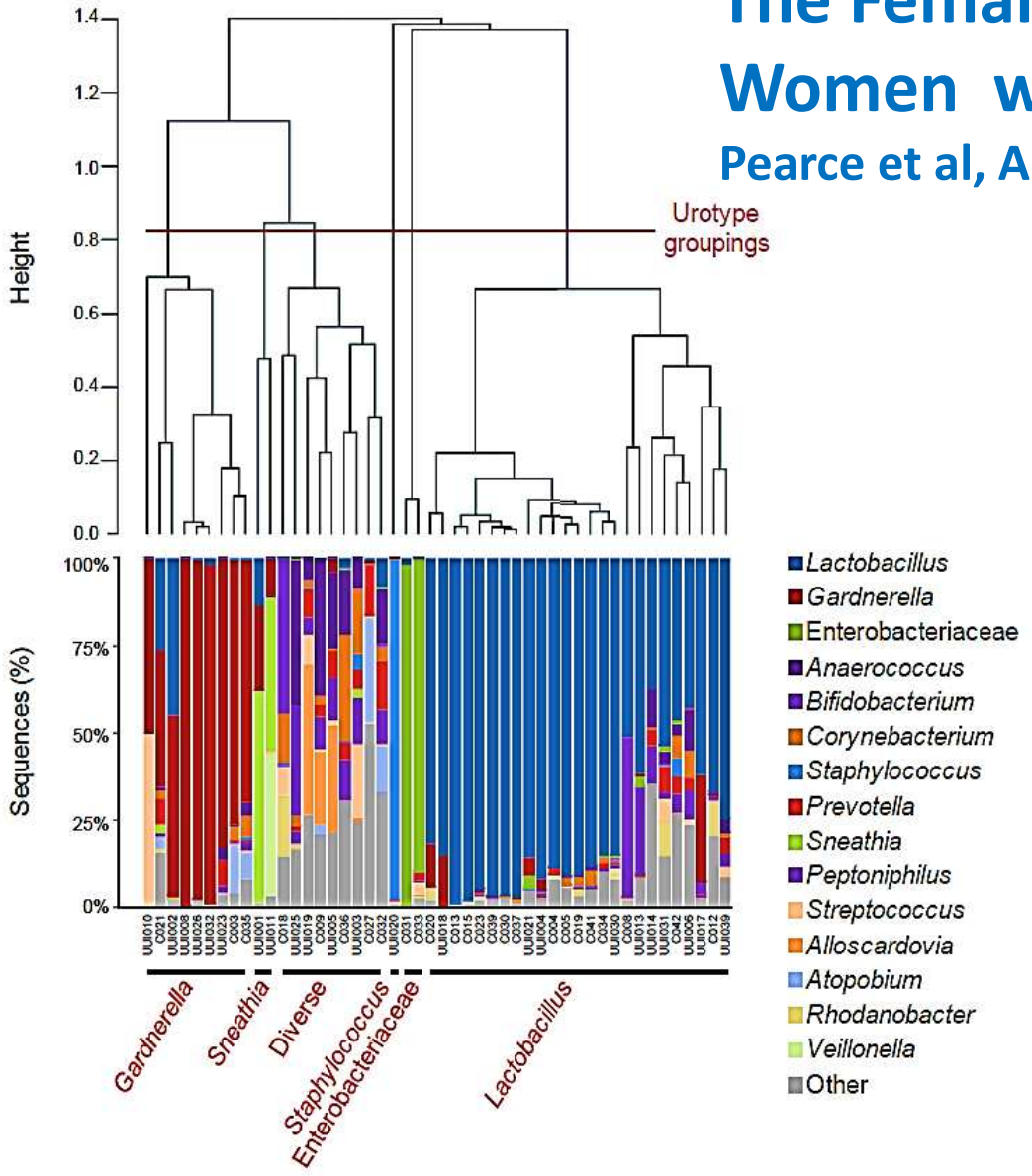


FIG 2 Clustering of the urinary microbiome into urotypes. The dendrogram was based on hierarchical clustering of the Euclidean distance between samples in the combined UUI and non-UUI cohorts. The dashed line depicts where the clades were divided into 6 urotypes: *Gardnerella*, *Sneathia*, Diverse, *Staphylococcus*, *Enterobacteriaceae*, and *Lactobacillus*. The stacked bar plot below the dendrogram depicts the sequence abundances of the overall most abundant taxa.

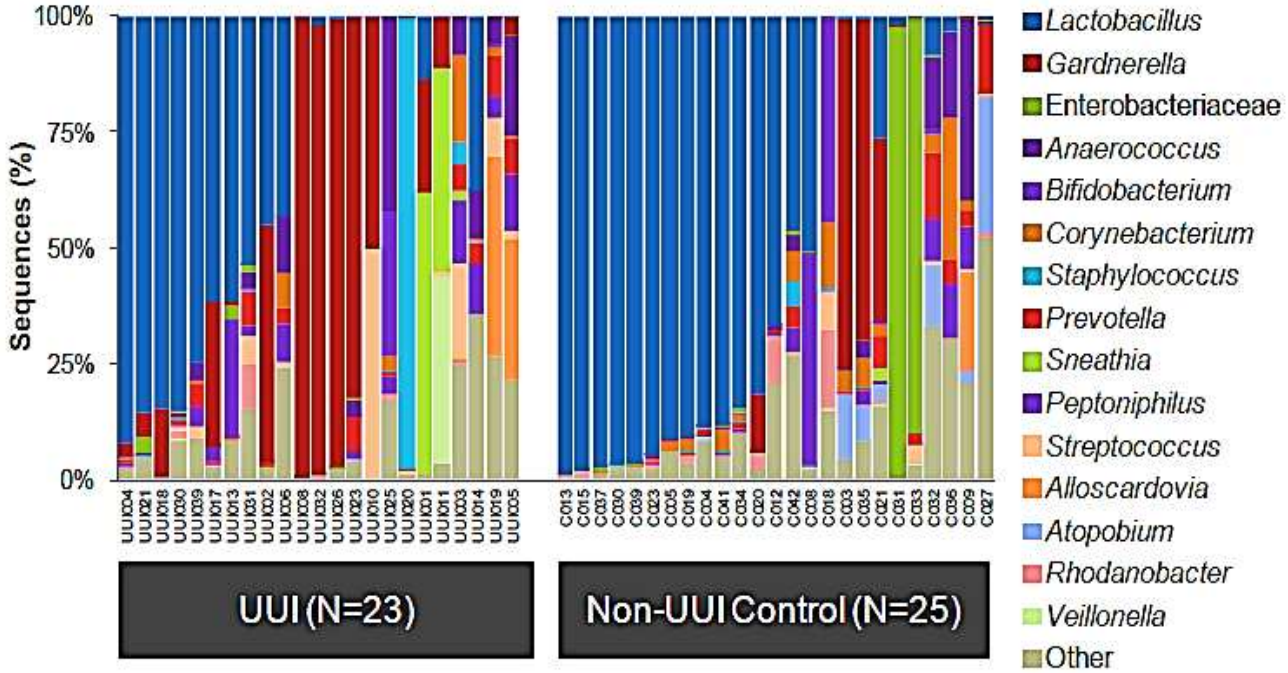
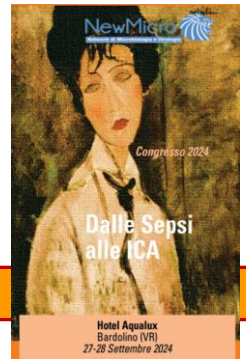


FIG 1 Urinary microbiome profile by cohort based on 16S rRNA gene V4 sequencing. Stacked bar plots depict the sequence abundances of the 15 most abundant genus- or family-level taxa in the UUI and non-UUI cohorts. Taxa were ranked according to mean abundance across all samples. The *y* axis represents the percentage of sequences for a particular bacterial taxa; the *x* axis represents the study participants separated by cohort. The family *Enterobacteriaceae* could not be classified to the genus level. The remainder of sequences were combined in the category labeled Other.



Linee Guida nell'adulto

SIGN
Scottish Intercollegiate Guidelines Network

A national clinical guideline

Updated July 2012

SIGN 88

Management of suspected bacterial urinary tract infection in adults

eau
European Association of Urology

Guidelines on Urological Infections

© European Association of Urology 2013

Linee Guida nel bambino

Urinary tract infection in children
Diagnosis, treatment and long-term management
Issued: August 2007 NICE clinical guideline 54

Urinary tract infection in infants, children and young people under 16
Issued: July 2013 NICE quality standard 36

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American Academy of Pediatrics
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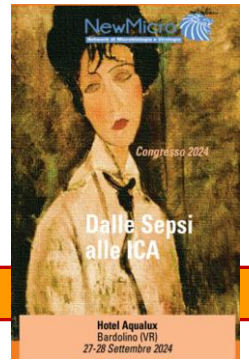
The New American Academy of Pediatrics Urinary Tract Infection Guideline
Thomas B. Newman
Pediatrics 2011;128;572; originally published online August 28, 2011;
DOI: 10.1542/peds.2011-1818

NICE National Institute for Health and Care Excellence

eau
European Association of Urology



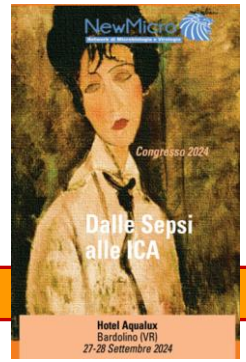
DE GRUYTER Clin Chem Lab Med 2024; 62(9): 1653–1786

Timo T. Kouri*, Walter Hofmann, Rosanna Falbo, Matthijs Oyaert, Sören Schubert, Jan Berg Gertsen, Audrey Merens and Martine Pestel-Caron, on behalf of the Task and Finish Group for Urinalysis (TFG-U), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

The EFLM European Urinalysis Guideline 2023

We aim to improve accuracy of urine examinations in European clinical laboratories, and to support diagnostic industry to develop new technologies.

Specimen collection: High-quality urine collection and preservation are supported with two quality indicators: contamination rate (cultures), and density of urine (chemistry, particles).



Linee Guida GIAU

SIBioC DOCUMENTS

biochimica clinica, 2011, vol. **35**, n. 2

DOCUMENTI SIBioC

L'esame chimico, morfologico e colturale delle urine: proposta di linea guida per una procedura standardizzata della fase preanalitica

Fabio Manoni[1], Alberta Caleffi[2], Gianluca Gessoni[3], Maria Grazia Alessio[4], Giuseppe Lippi[2], Sara Valverde[3], Cosimo Ottomano[4], Maria Grazia Silvestri[5], Piero Cappelletti[6], Mauro Ercolin[1], Michele Schinella[7], Graziella Saccani[8] per il Gruppo di Studio Intersocietario SIBioC-SIMeL Esame Urine

SIBioC DOCUMENTS

biochimica clinica, 2016, vol. **40**, n. 4

DOCUMENTI SIBioC

Esame fisico, chimico e morfologico delle urine: proposta di linee guida per la fase analitica del Gruppo Intersocietario Analisi delle Urine (GIAU)[a]

Fabio Manoni[1], Gianluca Gessoni[2], Giovanni Battista Fogazzi[3], Maria Grazia Alessio[4], Alberta Caleffi[5], Giovanni Gambaro[6], Maria Grazia Epifani[7], Barbara Pieretti[8], Angelo Perego[9], Cosimo Ottomano[10], Graziella Saccani[11], Sara Valverde[2], Sandra Secchiero[7]

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La Rivista Italiana della Medicina di Laboratorio 2019 Marzo;15(1):46-59
DOI: 10.23736/S1825-859X.19.00008-2

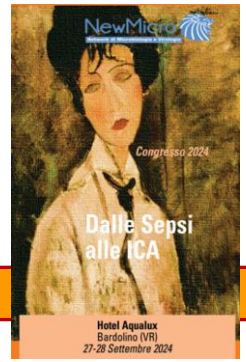
RACCOMANDAZIONI E LINEE GUIDA

Esame fisico, chimico e morfologico delle urine: raccomandazioni per la fase postanalitica del Gruppo Interdisciplinare Laboratorio e Clinica Apparato Urinario (GIAU)

Physical, chemical and morphological urine examination: recommendations for the postanalytical phase from the Interdisciplinary Urinalysis Group (GIAU)

Fabio MANONI [1] *, Gianluca GESSONI [2], Giovanni B. FOGAZZI [3], Maria G. ALESSIO [4], Rudi RAVASIO [4], Alberta CALEFFI [5], Giovanni GAMBARO [6], Sandra SECCHIERO [7], Barbara PIERETTI [8], Cosimo OTTOMANO [9], Anna LIVERANI [1], Cettina DRAGO [10], Fiamma BALBONI [11], Maria G. EPIFANI [7], Graziella SACCANI [12], Giovanni DI RIENZO [13], Sara VALVERDE [2], Giuliano BRUNORI [14], Loreto GESUALDO [15] a nome del Gruppo Interdisciplinare Analisi delle Urine (GIAU) ‡

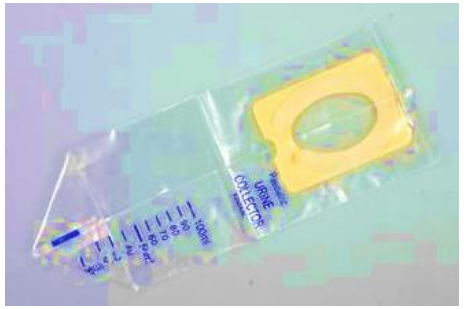
GIAU



Modalità di raccolta

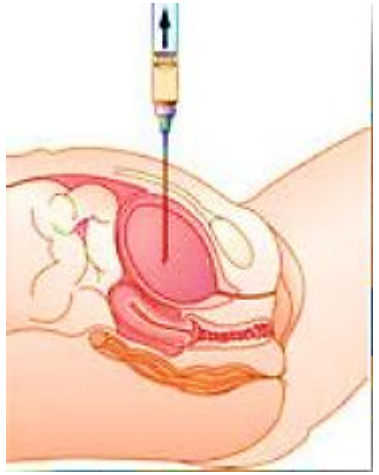
- Mitto intermedio

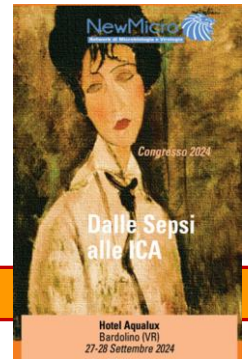
- Sacchetto sterile perineale



- Catetere

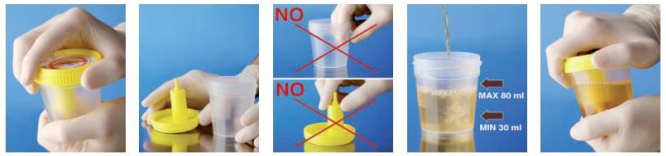
- Puntura sovrapubica





Istruzioni per la raccolta/ Idoneità del campione

URINE CONTAINER WITH COLLECTION DEVICE / CONTENITORE PER URINA CON DISPOSITIVO DI PRELIEVO



1. Attention: for microbiological tests clean the hands and genitals thoroughly before use. OPEN THE CAP BY UNSCREWING ANTI-CLOCKWISE.
1. Attenzione: per analisi microbiologiche lavare accuratamente mani e genitali esterni. TOGLIERE IL TAPPO SVITANDO IN SENSO ANTIORARIO.

2. LAY THE CAP UPSIDE DOWN ON A CLEAN SURFACE. 2. APPOGGIARE SU UNA SUPERFICIE PULITA IL COPERCHIO CAPOVOLTO.

3. DO NOT TOUCH INTERNAL SURFACES OF THE CONTAINER AND CAP.
3. NON TOCCARE LE SUPERFICI INTERNE DEL CONTENITORE E DEL COPERCHIO.

4. COLLECT THE URINE SAMPLE. Fill the container up to ¾ of the capacity.
4. RACCOGLIERE IL CAMPIONE DI URINE. Riempire il contenitore per non più di ¾ della sua capacità.

5. TURN THE CAP TIGHTLY IN A CLOCKWISE DIRECTION TO SEAL.
5. RICHIUDERE ACCURATAMENTE IL CONTENITORE AVVITANDO IL COPERCHIO IN SENSO ORARIO.

6. GENTLY SHAKE THE SAMPLE BEFORE TRANSFERRING IT TO THE TUBE.
6. OMOGENEIZZARE IL CAMPIONE PRIMA DEL TRASFERIMENTO IN PROVETTA.

7. PARTIALLY RAISE THE PROTECTIVE LABEL (DO NOT remove it completely).
7. SOLLEVARE PARZIALMENTE L'ETICHETTA PROTETTIVA (NON toglierla completamente).

8. INSERT THE TUBE. GENTLY APPLY PRESSURE. KEEP THE TUBE IMMERSED UNTIL IT IS FULL (end of flow).
8. INSERIRE LA PROVETTA. ESERCITARE E MANTENERE UNA MODERATA PRESSIONE SULLA PROVETTA. ATTENDERE FINO AL COMPLETO RIEMPIMENTO (cessazione del flusso).

9. REMOVE THE TUBE AND FULLY RESTICK THE PROTECTIVE LABEL.
9. TOGLIERE LA PROVETTA. RIPOSIZIONARE ACCURATAMENTE L'ETICHETTA PROTETTIVA SUL COPERCHIO.

10. TUBES WITH PRESERVATIVE GENTLY SHAKE THE SAMPLE 8-10 TIMES.
10. PROVETTE CON CONSERVANTE: OMOGENEIZZARE IL CAMPIONE (8-10 VOLTE per inversione lenta)

RACCOLTA URINE PER URINOCOLTURA IN PAZIENTI PEDIATRICI

1. Lavare accuratamente le mani con acqua e sapone
2. detergere accuratamente i genitali esterni
3. applicare il sacchetto sterile (reperibile in farmacia) facendolo aderire bene alla cute
4. lasciare "in situ il sacchetto per **non più di 60-90 minuti; se necessario provvedere alla sua sostituzione e ripetere l'operazione**
5. appena avvenuta la minzione,rimuovere il sacchetto, richiuderlo accuratamente,riporlo **chiuso senza travasare** in un contenitore in plastica sterile per urinocoltura e consegnare nel più breve tempo possibile al laboratorio.

Azienda Ospedaliera Ospedale Niguarda Ca' Granda — Regione Lombardia

S.C. ANALISI CHIMICO-CLINICHE E MICROBIOLOGIA

ISTRUZIONI PER IL PAZIENTE

IOP.ACCM.CPOsam.30 Rev.11 del 10/09/2014 Pag. 1 di 1

ISTRUZIONI PER URINOCOLTURA

Per la raccolta procurarsi presso il Centro Prelievi Ospedaliero (padiglione 9 piano terra) una provetta con tampone **UriSWAB tappo giallo**

ISTRUZIONI PER LA RACCOLTA DEL CAMPIONE:

- la raccolta del campione deve avvenire il mattino del giorno della consegna dell'esame, raccogliendo le urine almeno dopo due ore dall'ultima minzione
- lavare i genitali con acqua e sapone
- aprire la provetta. **Non** toccare il tampone e la spugnetta.
- iniziare ad urinare scartando la prima parte del getto.
- urinare **direttamente** sulla spugnetta ;
- inserire il tampone con la spugnetta nella provetta e riavvitare bene il tappo
- non toccare l'interno del contenitore, non inserire urina nella provetta
- consegnare il campione presso il **Centro Prelievi Ospedaliero, ritirando il numero al Distributore Automatico Eliminacode dalle 7.30 alle 14.45**

Pur essendo le precedenti modalità quelle ideali per la raccolta, tuttavia non costituiscono in alcun modo una necessità assoluta per la corretta esecuzione del test. A questo scopo è necessario unicamente che il paziente non abbia urinato da almeno 2 ore. La raccolta può anche essere eseguita seduta stante presso il Centro Prelievi Ospedaliero, qualora il paziente non abbia provveduto a raccogliere il campione a casa o qualora il campione raccolto sia, per qualsiasi motivo, non accettabile.

URINOCOLTURA PER RICERCA MICOBATTERI/BACILLO DI KOCH

Procurarsi contenitore sterile con tappo a vite a bocca larga.

- la raccolta del campione deve avvenire il mattino raccogliendo le urine almeno dopo due ore dall'ultima minzione
- lavare i genitali con acqua e sapone
- aprire la provetta. **Non** toccare l'interno del contenitore.
- iniziare ad urinare scartando la prima parte del getto.
- Raccogliere almeno 40 mL di urine (accertarsi che il contenitore sia quasi pieno).
- Chiudere riavvitando bene il tappo

Raccogliere, seguendo le modalità descritte precedentemente, 3 campioni di urine in giorni diversi.
Perché l'esame venga eseguito correttamente, è preferibile che ciascun campione venga consegnato nella stessa giornata di raccolta. Se non possibile la consegna in giornata, conservare i campioni a temperatura di frigorifero (2- 8 °C), fino alla consegna.

URINOCOLTURA PER BAMBINI FINO A DUE ANNI

La raccolta può essere eseguita con sacchetto in plastica sterile adesivo per bambini piccoli dove non sia possibile la raccolta di urina come sopra descritto sotto la supervisione di personale addestrato.
Dopo la raccolta delle urine il sacchetto deve essere posto in un contenitore sterile con tappo a vite. Sacchetto e contenitore sterili possono essere ritirati presso il Centro Prelievi Ospedaliero.

Questa modalità di prelievo ha un'elevata probabilità di contaminazione. Proprio per questa ragione, mentre un risultato negativo rende improbabile la presenza di una infezione urinaria, un eventuale risultato positivo non indica automaticamente che il bambino non abbia una infezione urinaria ma piuttosto che è necessario procedere ad ulteriori accertamenti e ad un prelievo delle urine mediante cateterismo vescicale secondo il giudizio del pediatra.

zaf — **PREPARAZIONE RACCOLTA URINE PER ESAME URINE COMPLETO E URINOCOLTURA** — cod. S92 data 09/11/16 rev. 3

Gentile Signora/e

La informiamo che per eseguire l'esame è importante attenersi alle istruzioni di seguito riportate.

PREPARAZIONE ALLA RACCOLTA DEL CAMPIONE DI URINE

- Utilizzare un contenitore sterile reperibile in farmacia
- Devono essere emesse le prime urine del mattino, dopo il riposo notturno
- Utilizzare sapone/salviettine detergenti disinfettanti o normali per l'igiene intima e salviette in carta monouso o garze.

Istruzione per le donne:

1. Lavarsi accuratamente le mani con acqua e sapone
2. lavarsi bene i genitali esterni utilizzando una soluzione saponosa, allargando il più possibile le ginocchia, divaricando con una mano le grandi labbra; mantenendole divaricate durante le operazioni di detersione e raccolta delle urine
3. lavarsi dall'avanti all'indietro
4. risciacquarli con acqua, con lo stesso movimento dall'avanti all'indietro
5. asciugarsi usando una per volta, salviette o garze scartandole dopo ogni passaggio,
6. aprire il contenitore e tenerlo con le dita sulla parete esterna senza toccare il bordo né con le dita né con la cute, urinare eliminando il primo getto e senza interrompere la minzione, raccogliere il mitto intermedio direttamente nel contenitore
7. fare attenzione che il getto di urina non coli sulla cute, ma che sia ben diretto al contenitore
8. chiudere il contenitore con attenzione e accertarsi che non fuoriesca alcun liquido.

Istruzione per i maschi:

1. Lavarsi accuratamente le mani con acqua e sapone
2. lavarsi accuratamente e ripetutamente i genitali esterni dopo aver retratto completamente la cute sul glande utilizzando una soluzione saponosa
3. risciacquarli abbondantemente con acqua
4. asciugarsi usando salviette pulite
5. aprire il contenitore e tenerlo con le dita sulla parete esterna senza toccare il bordo né con le dita né con la cute, urinare eliminando il primo getto e senza interrompere la minzione, raccogliere il mitto intermedio direttamente nel contenitore
6. fare attenzione che il getto di urina non coli sulla cute, ma che sia ben diretto al contenitore;
7. chiudere il contenitore con attenzione

CONSEGNA CAMPIONE

Il contenitore deve essere conservato in frigorifero fino alla consegna presso il Punto Prelievi negli orari di apertura.

Compilazione	Verifica	Approvazione
Direzione di Laboratorio	Assicurazione Qualità	Direzione Sanitaria
Dott.ssa Laura Soldini	Dott.ssa Manuella Ghezzi	Dott. Gabriele Pelliccioni

Regione Lombardia — ASL Bergamo — **ASL DELLA PROVINCIA DI BERGAMO** COORDINAMENTO DIREZIONI SANITARIE DELLE STRUTTURE OSPEDALIERE PUBBLICHE E PRIVATE ACCREDITATE — HUMANITAS

URINOCOLTURA
(Urine da mitto intermedio)

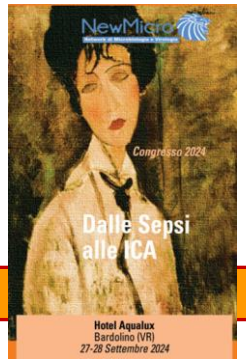
CHE MATERIALE SERVE PER LA RACCOLTA?
Contenitore sterile da 100-200 ml con apertura larga, con tappo a vite e con etichetta; viene consegnato dal Laboratorio/Centro Prelievi o si può comprare in Farmacia.

COME RACCOGLIERE LE URINE?
Può raccogliere **le prime urine emesse al risveglio, oppure quelle successive, solo se sono passate almeno 2 ore dall'ultima volta che ha urinato.**

1. **Prima della raccolta lavi bene** le mani e i genitali esterni con acqua e sapone (non usi disinfettanti!), **risciacqui e asciughi molto bene.**
   E' meglio raccogliere le urine a casa; se invece pensa di raccogliere le urine presso il Laboratorio/Centro Prelievi, deve lavarsi e fare il bidet di pulizia a casa. Poi al momento della raccolta, prima di urinare, deve lavare molto bene le mani.
2. **Apra il contenitore sterile senza toccarlo all'interno.**
3. **Scopra il glande (per gli uomini) o apra con le dita le labbra della vulva (per le donne)** per ridurre le possibilità di contaminazione dell'urina.
4. **Butti via il primo getto di urina. Raccolga le urine successive (mitto intermedio) nel contenitore sterile ed elimini le ultime urine.**
   Riempia non più della metà del contenitore.
5. **Chiuda subito il contenitore sterile**, senza toccarlo all'interno e avvitando bene il coperchio.
6. **Consegni le urine al Laboratorio/Centro Prelievi entro 2 ore** dalla raccolta. Se ciò non è possibile, può consegnare le urine **entro 24 ore, conservando il contenitore in frigorifero (non in congelatore!). Se nel contenitore c'è un conservante (es. Acido Borico), può tenere le urine a temperatura ambiente per 24/36 ore.**

Attenzione: se è richiesto anche **l'esame completo delle urine**, deve raccogliere parte del mitto intermedio in un altro contenitore pulito o in una provetta a fondo conico trasparente da 10 ml e consegnarla entro 2 ore al Laboratorio/Centro Prelievi.

RACCOMANDAZIONE: esame chimico-fisico + urinocoltura nel sospetto di UTI

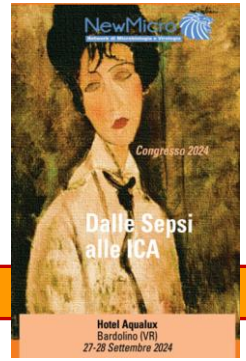


Diagnosi

I **sintomi** di una cistite cioè fastidio, dolore al basso ventre, necessità di urinare spesso, bruciore alla minzione, sono in genere sufficienti al medico per fare la diagnosi.

Gli esami di **laboratorio** utilizzati per la conferma sono:
l'**esame delle urine** (presenza di globuli bianchi, batteri e nitriti; in alcuni casi globuli rossi)

l'**urinocoltura** che permette di isolare il germe responsabile dell'infezione
e di verificarne la risposta a diversi antibiotici (**antibiogramma**).



Una proposta di protocollo per la gestione dell'esame microbiologico delle urine: come garantire l'appropriatezza riducendo il lavoro improduttivo

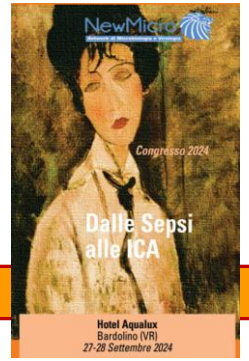
A. Camporese, Riv Med Lab–JLM, Vol 12:54-58, 2016

No screening per pazienti in **gravidanza** e nei reparti/strutture a cui afferiscano pazienti **immunocompromessi** (es. oncoematologia, centri trapianti).

Screening strumentale per batteri e leucociti:

1. Positivo → urinocoltura
2. Negativo → negativo allo screening o non appropriato per esame colturale

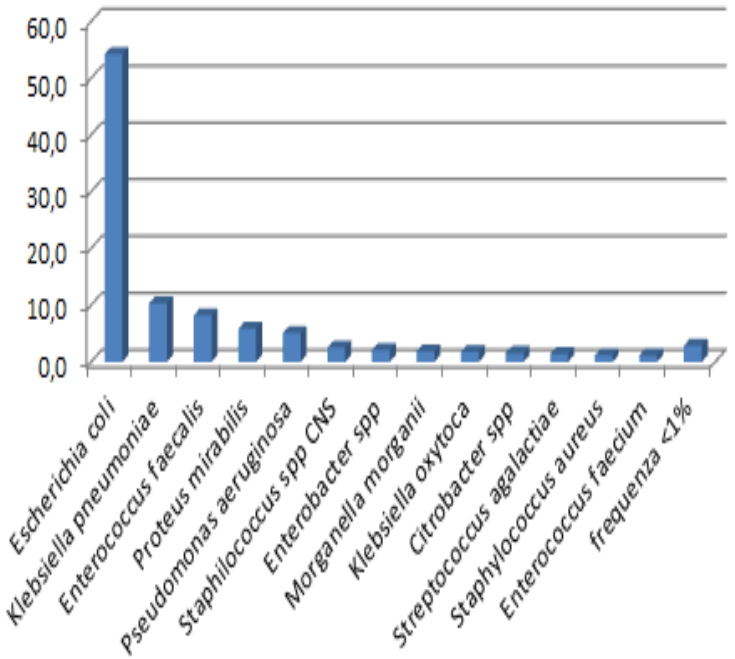
Per garantire l'appropriatezza clinica della risposta: esame microbiologico delle urine → esame colturale + quantificazione degli elementi cellulari in grado di definire la significatività clinica del campione inviato (leucociti) e/o la presenza di eventuale contaminazione perineale (cellule epiteliali squamose).

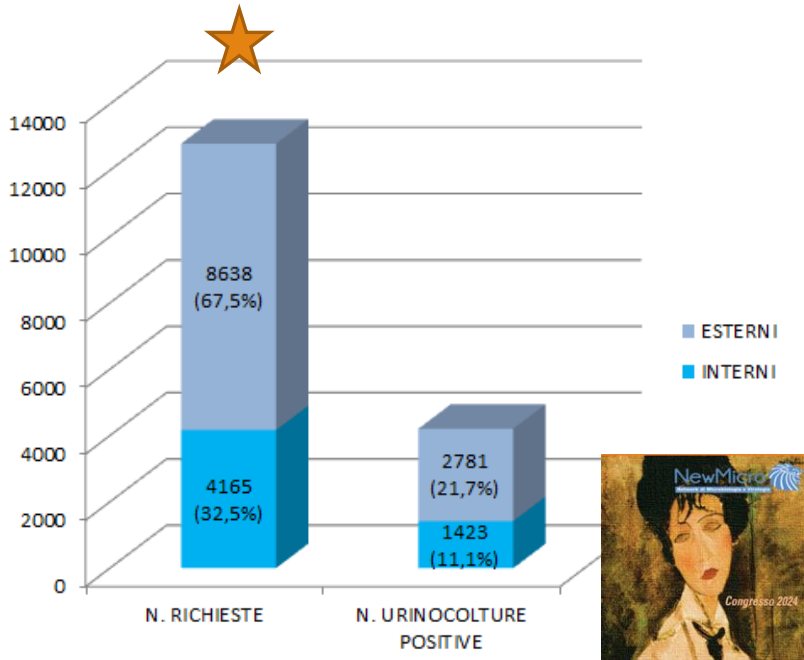


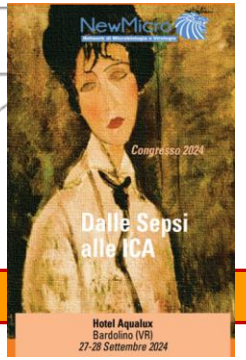
2016	N.Richieste	Osservazioni
Esame urine	83.993	82.185 (97.9%) routine
Urinocoltura	12.803	4.204 (32.8%) positive

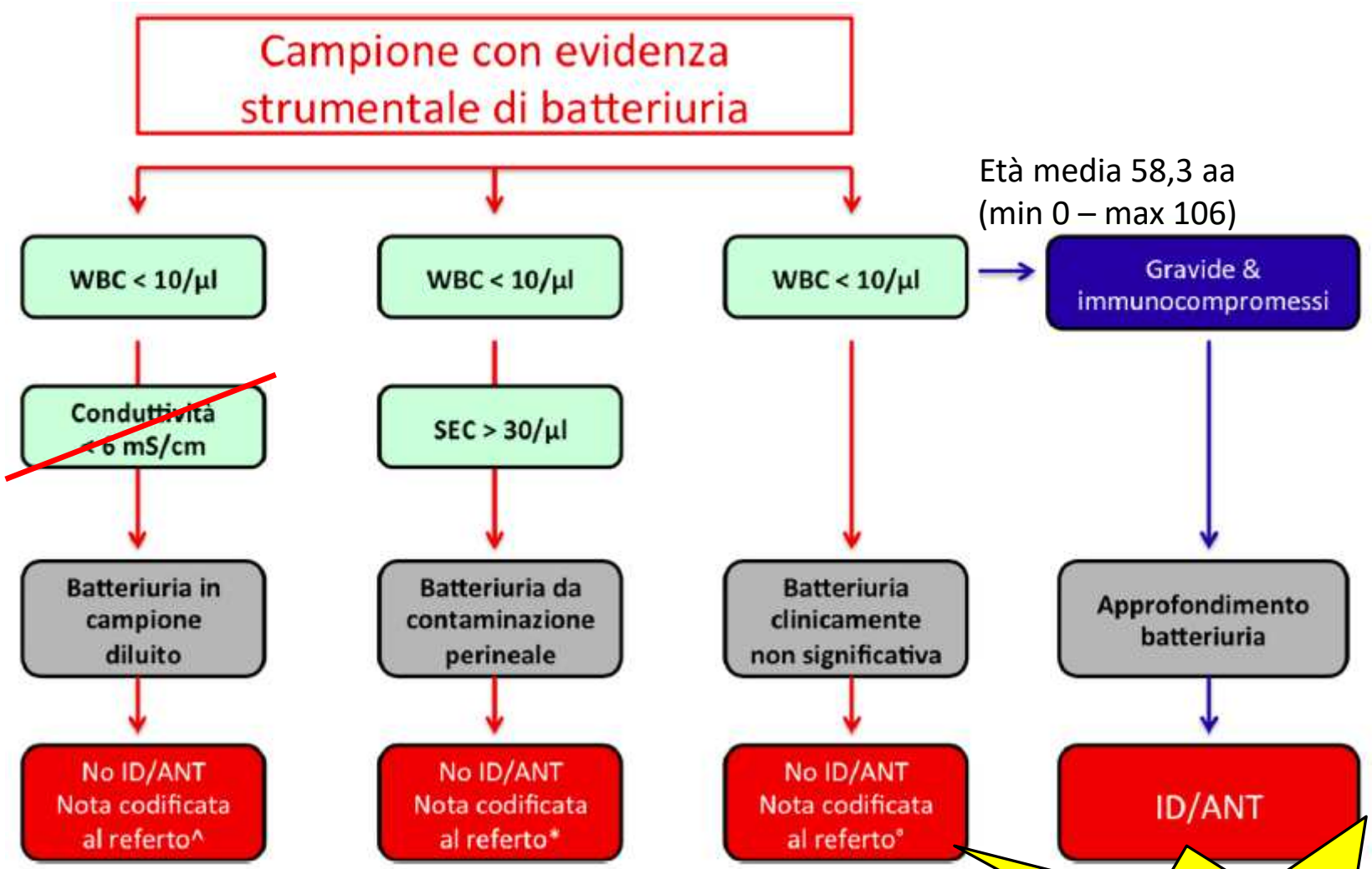
Esame urine	Tot.	%
Batteri	14233	16.9
Cellule epiteliali	6603	7.9
Cellule epiteliali+ Esterasi	3121	3.7
Cellule epiteliali + Leucociti	4918	5.9
Cellule epiteliali + Leucociti + Batteri	2773	3.3

Microrganismi	Tot.	%
Escherichia coli	2279	54,2
Klebsiella pneumoniae	435	10,3
Enterococcus faecalis	344	8,2
Proteus mirabilis	246	5,9
Pseudomonas aeruginosa	213	5,1
Staphilococcus CNS	106	2,5
Enterobacter spp	88	2,1
Morganella morganii	78	1,9
Klebsiella oxytoca	74	1,8
Citrobacter spp	66	1,6
Streptococcus agalactiae	59	1,4
Staphylococcus aureus	48	1,1
Enterococcus faecium	47	1,1
Frequenza <1%	121	2,9



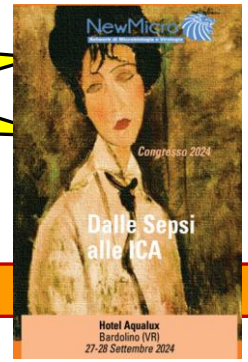






Es. urine	1.8%	0.6%	3.4%
Es. urine + coltura pos (1688 campioni)	8.7%	0.5%	8.7%

No WBC, CE,BATT 59.4%



L'urinocoltura potrebbe essere un test riflesso a fronte di un test di screening automatizzato positivo ?

Pieretti Barbara[1], Forconi Giuliana[2], Brecciaroli Francesca[2], Secondini Sara[2], Giampaoli Alessandra[2], Moretti Marco[1], Pauri Paola[2]

[1] UO Patologia Clinica - Azienda Ospedaliera Ospedali Riuniti Marche Nord Fano [2] UOC Patologia Clinica - Ospedale Jesi - Area Vasta 2

AMCLI XLIII° Congresso Nazionale Rimini 04-07 novembre 2014

Lo scopo dello studio è stato quello di verificare le potenzialità diagnostiche del sistema Iris iQ®200 (Beckman Coulter) nello screening delle batteriurie, confrontandole con il metodo di riferimento rappresentato dall'urinocoltura, per ottenere un miglioramento dei flussi operativi relativi alle urinocolture e della gestione delle infezioni delle vie urinarie (IVU) all'interno del laboratorio.

Jesi — ambito territoriale 21 Comuni, bacino di utenza 102.000 abitanti, territorio 673 Kmq

Fano — ambito territoriale 22 Comuni, bacino di utenza 125.000 abitanti, territorio 787 Kmq

DISEGNO DELLO STUDIO

American Academy of Pediatrics · SIGN · NICE

febbraio–giugno 2014 → 940

963 campioni consecutivi con richiesta di urinocoltura (in/out-patients)

stesso contenitore → semina urinocoltura (Mac Conkey/CNA)

→ un'aliquota per esame chimico-fisico e/o sedimento

confronto strumentale →

sedimento
WBC: CO ≥ 18/µL
Batteri: CO≥1/µL
elementi di piccole dimensioni: CO≥8000/µL

chimico-fisico
nitriti: CO ≥ 1
esterasi leucocitaria: CO ≥ 70/µL per Jesi
CO ≥ 25/µL per Fano

Un risultato positivo rispetto ai CO considerati per ognuno dei parametri indicati è considerato un test di screening positivo.

OSSERVAZIONI

Essendo i due ospedali territoriali di riferimento a bassa/media complessità il gold standard urinocoltura ha ottenuto una bassa percentuale di positività (31%) ed alta di negatività (69%), dimostrando sia un'**inappropriatezza della richiesta** che un eccessivo impiego di risorse umane e materiali a fronte di una scarsa resa diagnostica.

L'approccio sperimentato potrebbe evitare la semina di circa il 50% dei campioni negativi allo screening, oltre a consentire una **riduzione del TAT**, con immediata refertazione on-line dei negativi.

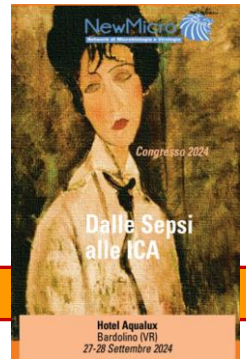
VPN = TN/(TN+FN)= **99,1%** — **VPP** = TP/(TP+FP)= **57,7%**

Sensibilità = VP/(VP+FN) = **98,6%** — **Specificità** = VN/(VN+FP) = **68,0%**

Accuratezza diagnostica =(VP+VN)/TOTALE = **77,3%**

urinocoltura come test riflesso di un esame urine patologico

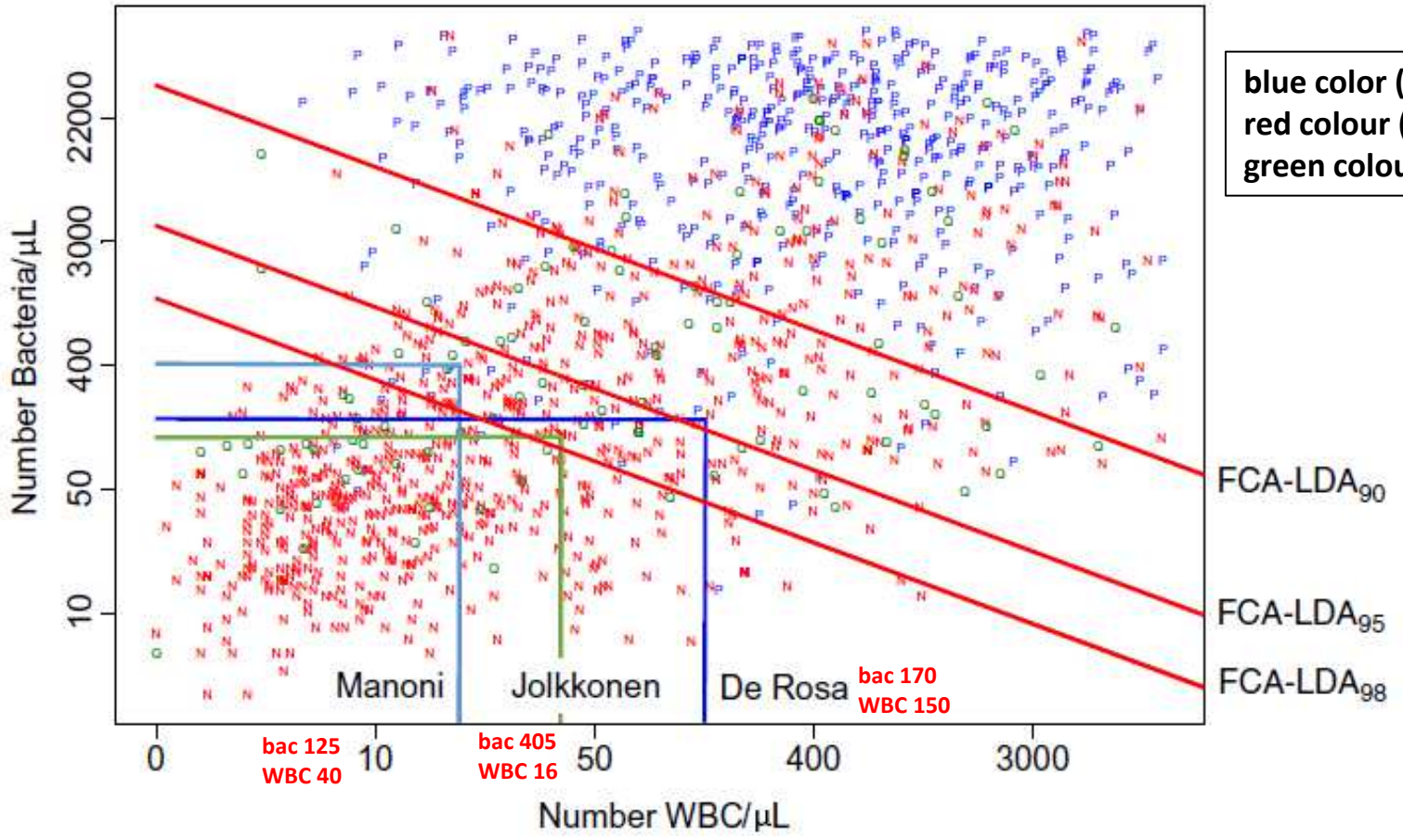
Migliorare l'efficienza del test di screening (↓ FP e FN), agire sulla fase preanalitica e proporre questo flusso di lavoro per ridurre i tempi di risposta, evitare l'inizio di una terapia antibiotica, contenere i costi (risorse umane ed economiche).



A new concept and a comprehensive evaluation of SYSMEX UF-1000i flow cytometer to identify culture-negative urine specimens in patients with UTI

T. Monsen et al, Eur J Clin Microbiol Infect Dis, DOI 10.1007/s10096-017-2964-1, 2017

The aim was to evaluate a new screening concept for flow cytometry analysis (FCA). The outcomes were evaluated against urine culture, uropathogen species and three conventional screening methods.



blue color (P) = positive specimens (significant bacteriuria)
red colour (N) = negative specimens (non-significant bacteriuria)
green colour (Q) = indeterminant/questionable specimens

red lines = cut off for linear discriminant analysis of sensitivity

We recommend the proposed screening method to be used in clinic to exclude culture negative specimens, to reduce workload, costs and the turnaround time.

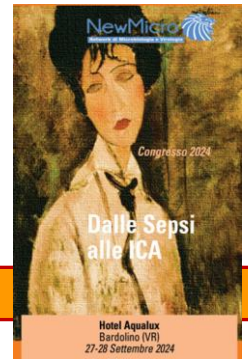


Table 3 Outcome of the four screening methods to rule out culture negative urine specimens among patients with suspected UTI

SBU[a]	Measures (%)	Methods evaluated[b]					
		Jolkkonen	Manoni	De Rosa	FCA-LDA$_{98}$	FCA-LDA$_{95}$	FCA-LDA$_{90}$
European Guidelines	SE	98	99	98	98	95	90
	SP	41	41	51	52	65	83
	PPV	51	52	56	56	63	77
	NPV	96	98	98	98	95	93
	NC[c]	26	25	32	32	42	56
	RC[d]	94	95	88	88	78	64
$\geq 10^7$ CFU/L	SE	94	96	94	94	90	81
	SP	42	42	53	52	67	84
	PPV	56	56	61	61	68	80
	NPV	91	93	92	92	89	85
$\geq 10^8$ CFU/L	SE	99	100	100	99	98	95
	SP	38	38	48	48	62	81
	PPV	44	44	49	49	56	71
	NPV	99	100	100	99	99	97

SE sensitivity, *SP* specificity, *PPV* positive predictive value, *NPV* negative predictive value

[a] SBU = Definition of significant bacteriuria according to European guidelines, $\geq 10^7$ and $\geq 10^8$ colony forming units per liter in urine at culture

[b] Comparing screening methods according to Jolkkonen et al., Manoni et al., De Rosa et al. Flow cell analysis-linear discriminant analysis at 98% sensitivity (FCA-LDA$_{98}$), FCA-LDA$_{95}$ = at 95% sensitivity, and FCA-LDA$_{90}$ = at 90% sensitivity

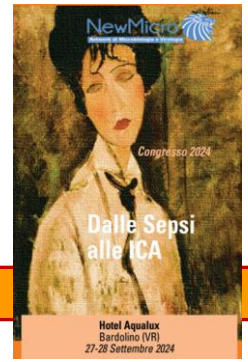
[c] NC = not cultured (%): specimens identified as culture negative by the screening method

[d] RC = relative cost of the screening method (%) compared to the gold standard procedure (100%) when all specimens are cultured. A calculated cost of, e.g. 78%, represents a 22% cost reduction when FCA screening was included in its cost

Flow citometry analysis
linear discriminant analysis (FCA-LDA)

A new concept and a comprehensive evaluation of SYSMEX UF-1000i flow cytometer to identify culture-negative urine specimens in patients with UTI

T. Monsen et al, Eur J Clin Microbiol Infect Dis, DOI 10.1007/s10096-017-2964-1, 2017



Flow citometry for screening acute urinary tract infections and differentiation between Gram positive and Gram negative bacteria

G. Saccani et al, biochimica clinica, Vol **38**, No 6:625-629, 2014

Table 4

Performance of flow citometry in discrimination between urinary tract infections sustained by Gram positive and Gram negative bacteria

	SE	SP	PPV	NPV	DA
B_FSC	0.68 (0.65-0.71)	0.89 (0.76-0.96)	0.38 (0.32-0.44)	0.91 (0.88-0.94)	0.71 (0.66-0.76)
B_FLH	0.57 (0.49-0.64)	0.65 (0.49-0.78)	0.24 (0.18-0.31)	0.87 (0.81-0.93)	0.54 (0.49-0.59)

Numbers in parentheses are 95% confidence intervals.

SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value; DA, diagnostic accuracy; B_FSC, bacteria forward scatter; B_FLH, bacteria fluorescent light scatter.

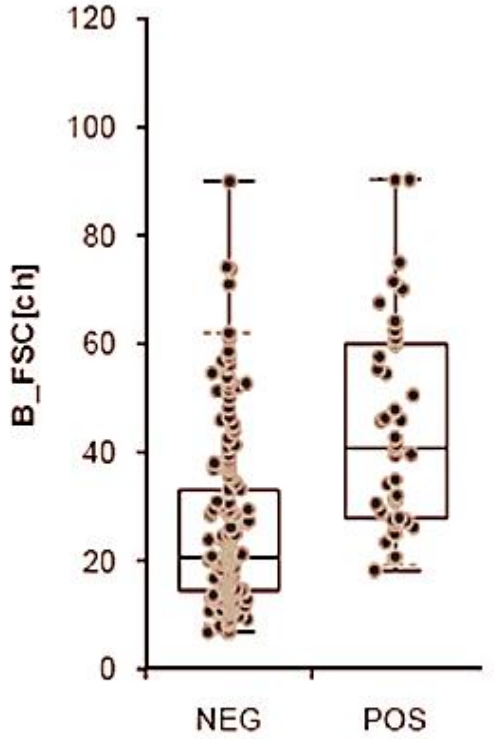
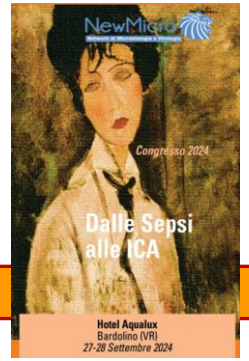


Figure 1

Bacteria forward scatter (B_FSC) in urinary tract infections sustained by Gram positive (POS) and Gram negative (NEG) bacteria .



Does flow citometry have a role in preliminary differentiation between urinary tract infections sustained by gram positive and gram negative bacteria? An Italian policentric study

G. Gessoni et al, Clinical chimica acta, Vol 440:152-156,2015

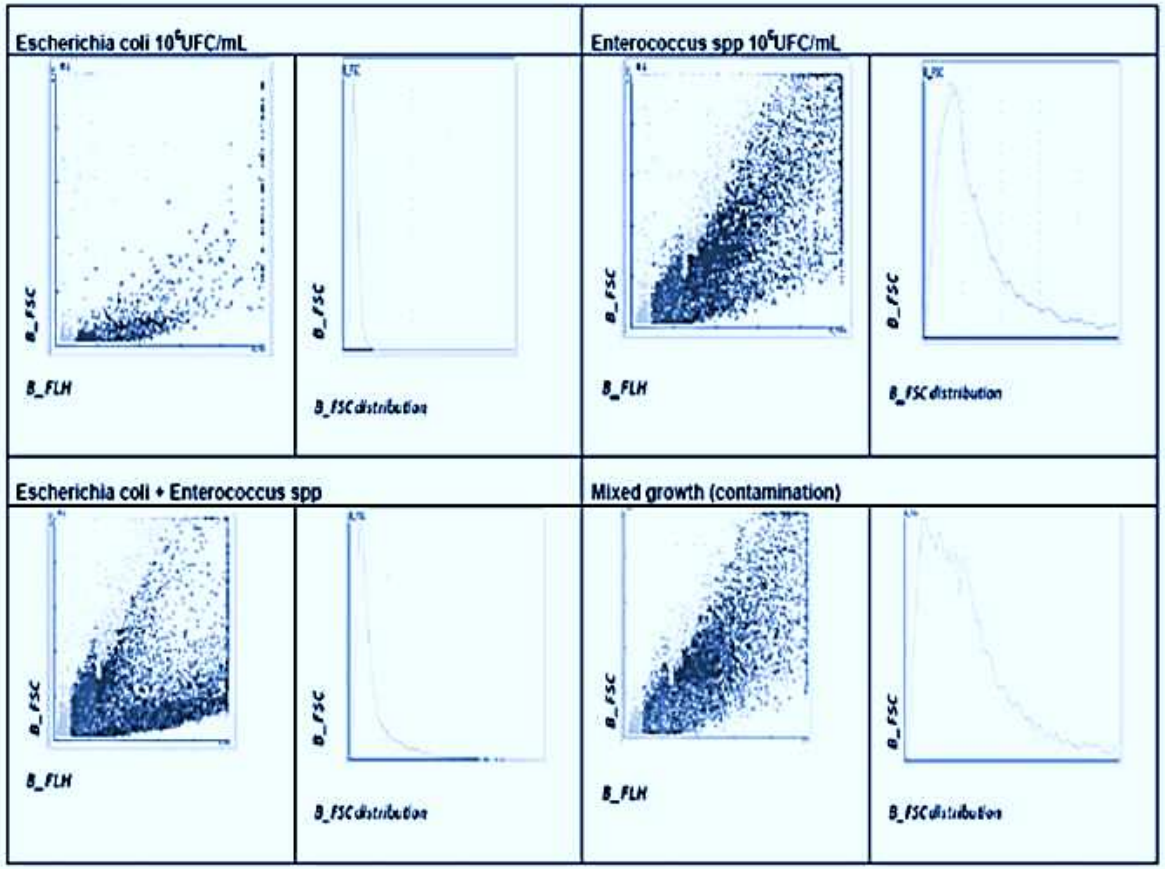
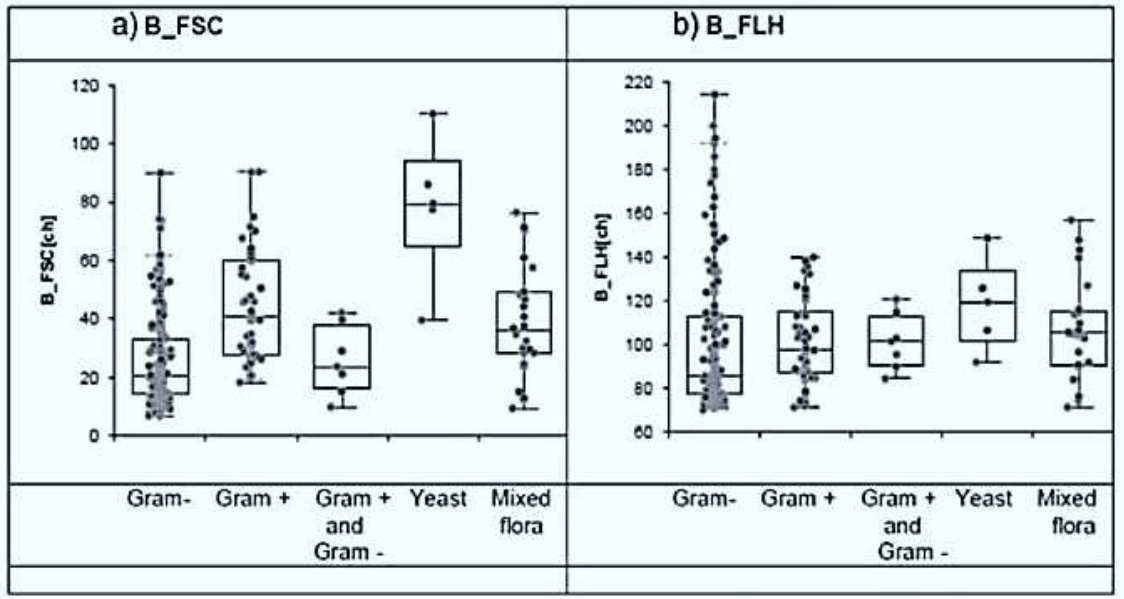
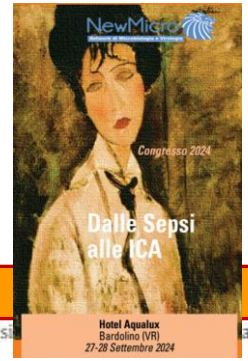


Fig. 1. Scattergrams for B_FSC versus B-FLH and ch. Gram neg: *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp.; Gram pos: *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp. B_FSC = bacteria forward scatter, B_FLH = bacteria fluorescent light scatter ch = arbitrary channel.





positive bacteria (119), an association of Gram positive and Gram negative bacteria (14), and Yeast (9). Moreover reported samples were consi ause of a polymicrobic growth (41).

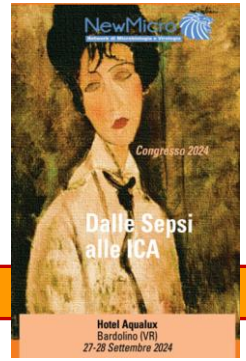
CONCLUSIONI

Microbiologo 2.0

L’esame microbiologico delle urine rappresenta un modo del tutto nuovo e clinicamente efficace di intendere la diagnostica microbiologica delle IVU.

Necessario agire migliorando la refertazione, attraverso l’utilizzo di commenti che invitino il clinico a valutare con maggiore attenzione la correlazione tra il risultato microbiologico e il contesto clinico, per evitare trattamenti inappropriati, oppure evitando di refertare i risultati espressione di contaminazione/ colonizzazione.

Esigenza di “consolidare” la diagnostica microbiologica con la diagnostica microscopica e chimico fisica.



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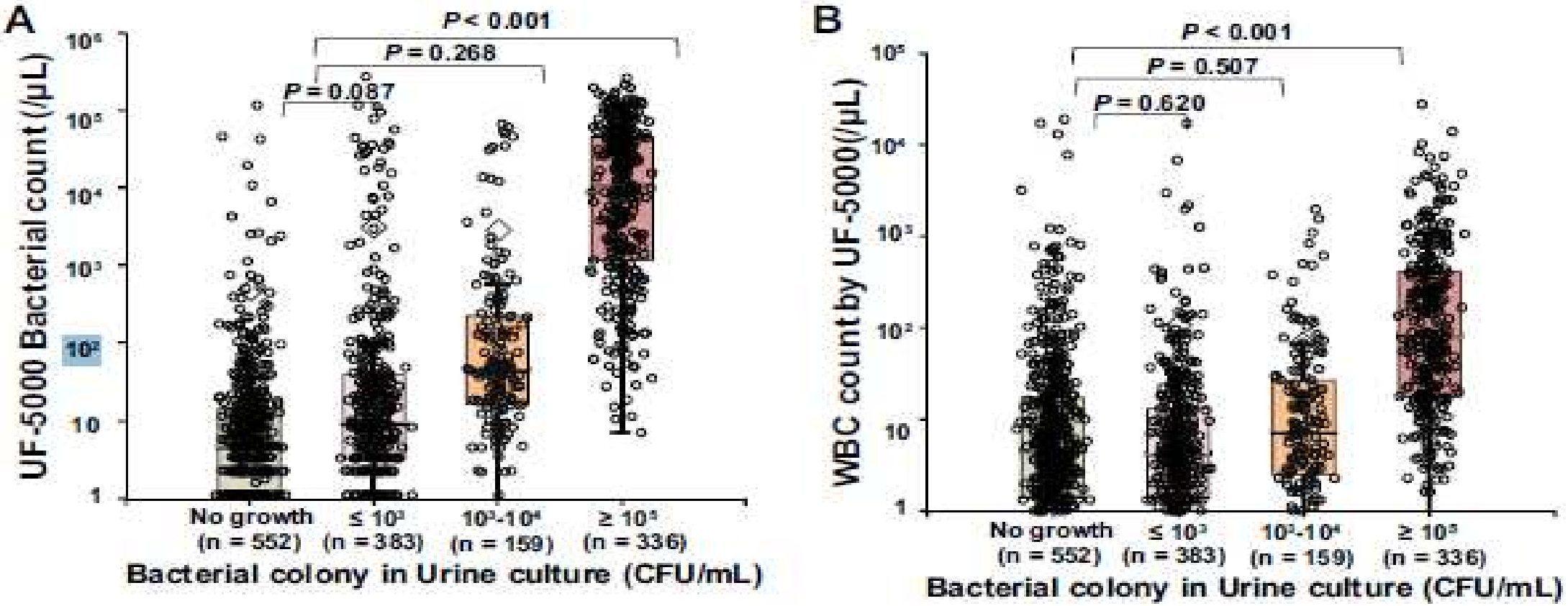
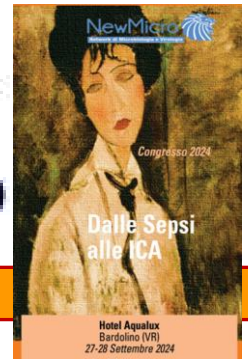
BACTERIOLOGY

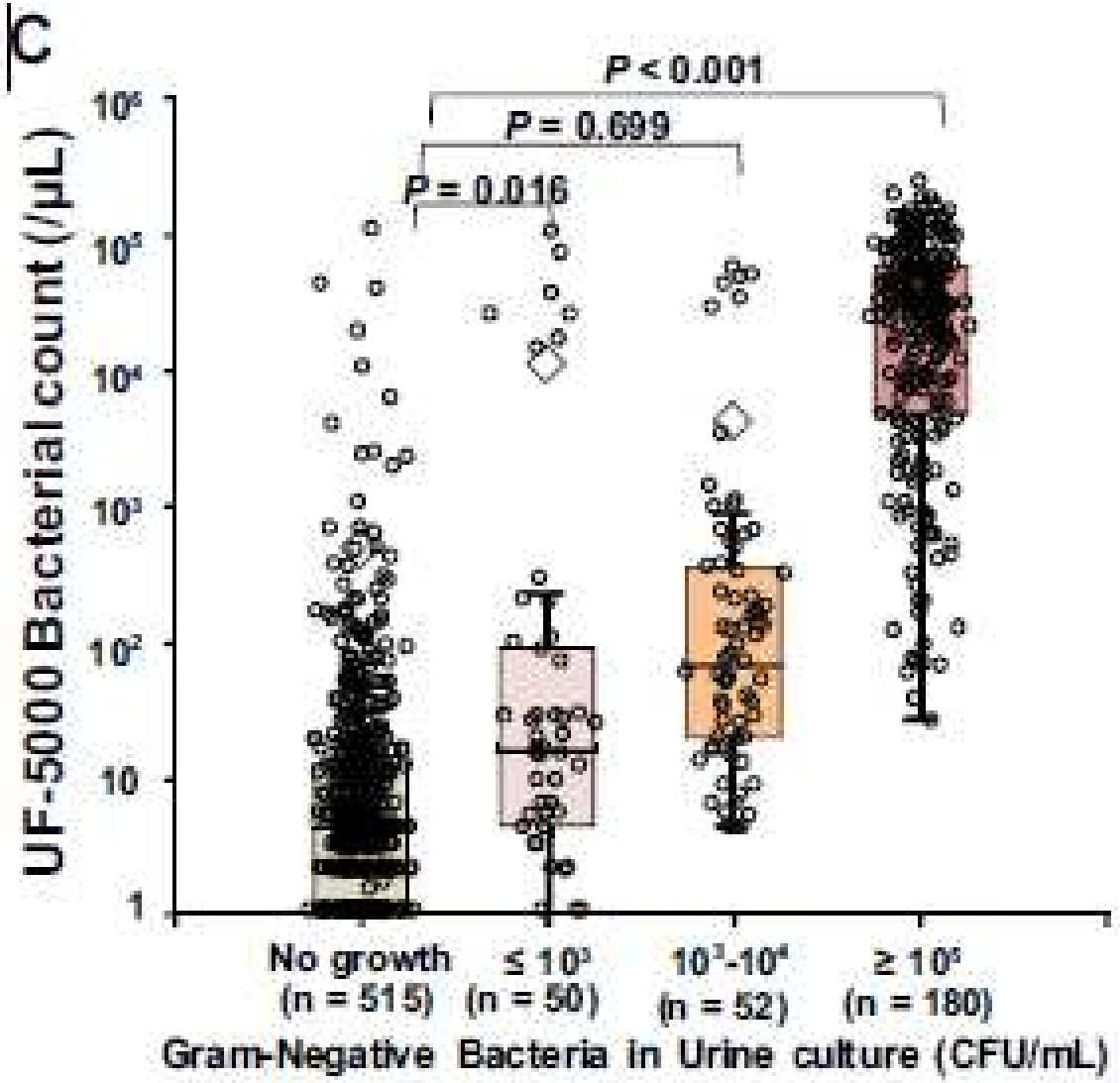
Rapid Screening of Urinary Tract Infection and Discrimination of Gram-Positive and Gram-Negative Bacteria by Automated Flow Cytometric Analysis Using Sysmex UF-5000

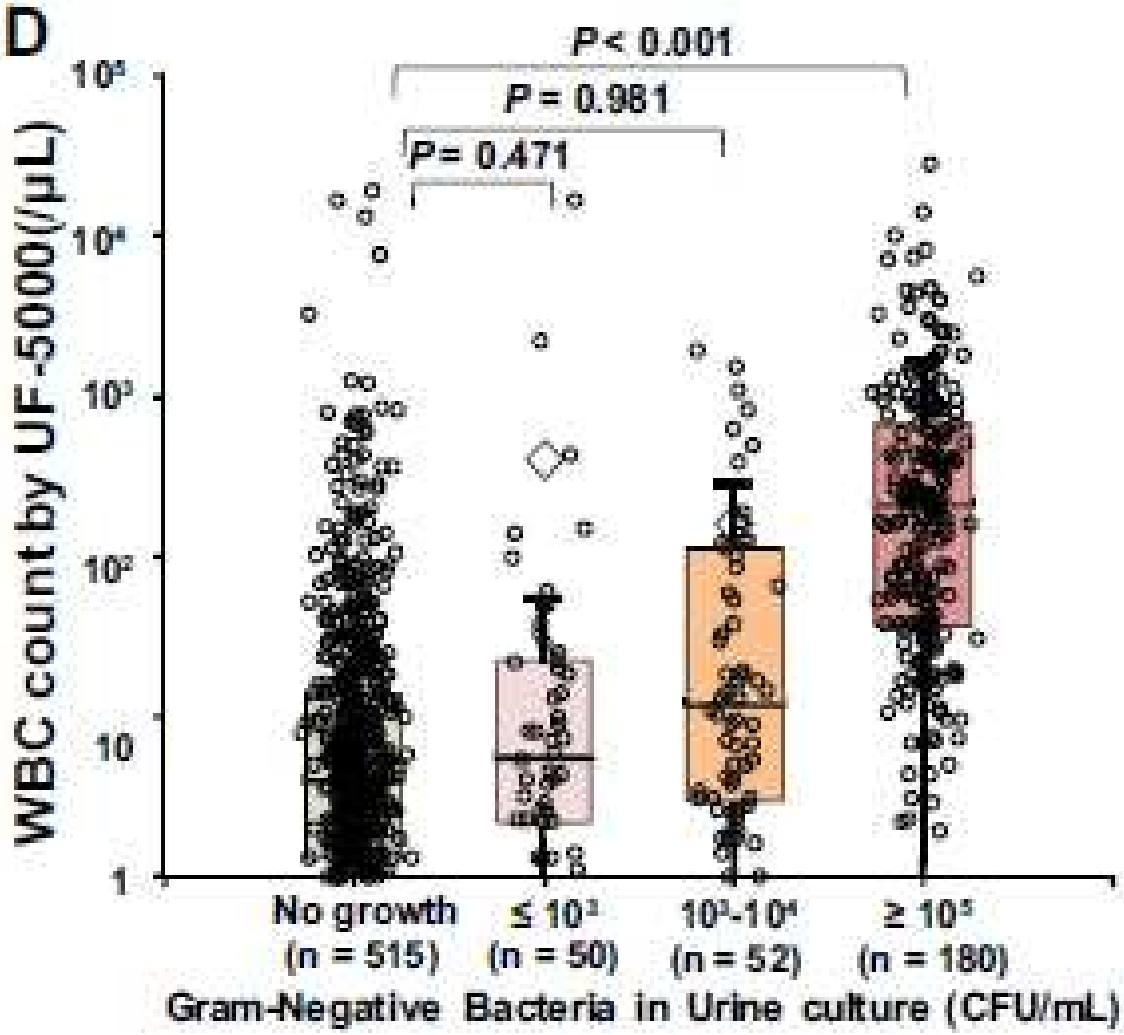
Seon Young Kim,[a] Yumi Park,[a] Hyunjin Kim,[a] Jimyung Kim,[a] Sun Hoe Koo,[a] Gye Cheol Kwon[a]

[a]Department of Laboratory Medicine, Chungnam National University School of Medicine, Chungnam National






C
P < 0.001
P = 0.699
P = 0.016
UF-5000 Bacterial count (/µL)
10⁵
10⁴
10³
10²
10
1
No growth (n = 515)
≤ 10³ (n = 50)
10³-10⁴ (n = 52)
≥ 10⁴ (n = 180)
Gram-Negative Bacteria in Urine culture (CFU/mL)


D
P < 0.001
P = 0.981
P = 0.471
WBC count by UF-5000(/µL)
10⁵
10⁴
10³
10²
10
1
No growth (n = 515)
≤ 10³ (n = 50)
10³-10⁴ (n = 52)
≥ 10⁴ (n = 180)
Gram-Negative Bacteria in Urine culture (CFU/mL)

NewMicro
Congresso 2024
Dalle Sepsi alle ICA
Hotel Aqualux
Bardolino (VR)
27-28 Settembre 2024

1.1 Examinations for general patient populations

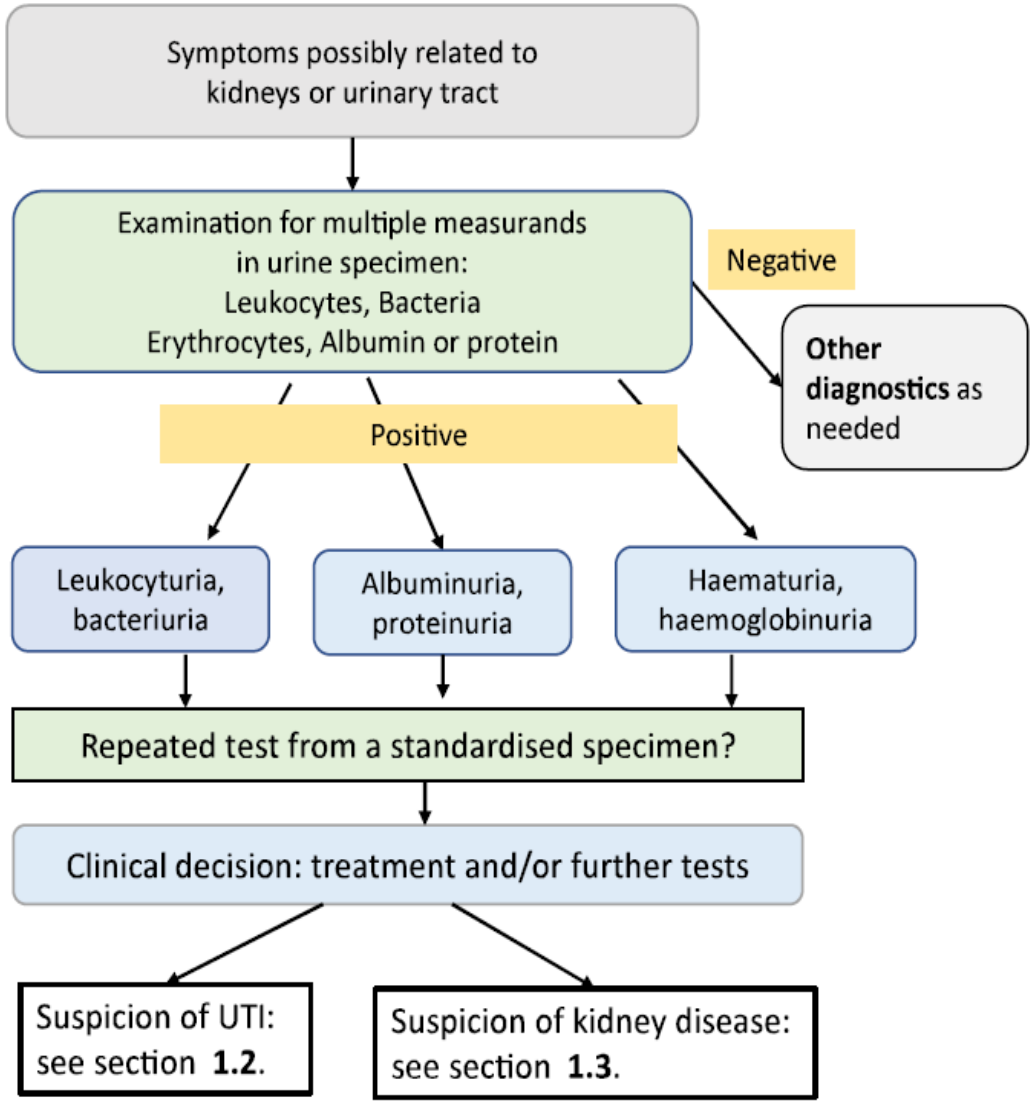


Figure 1: Urinalysis examinations with a sieving strategy for patients with general symptoms, possibly related to kidneys or urinary tract. The

1.2 Examinations for detection of urinary tract infection

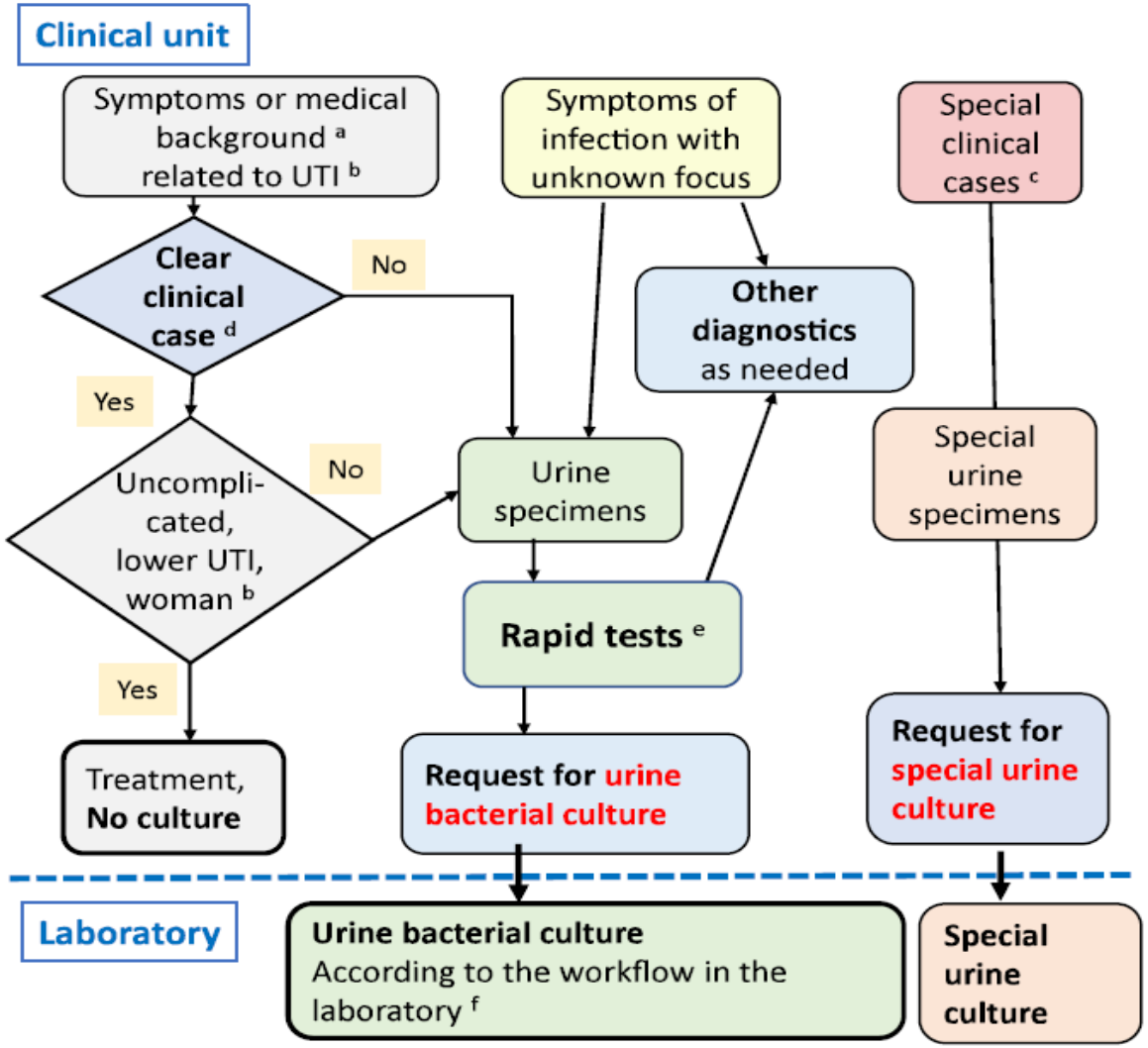
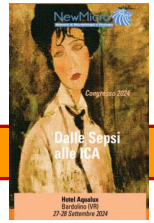


Figure 2: Urine examinations in suspicions of urinary tract infection. The

The EFLM European Urinalysis Guideline 2023



New and developing diagnostic technologies for urinary tract infections

Davenport M et al, Narure Reviews - Urology, http://dx.doi.org/10.1038/nrurol.2017.20, 2017

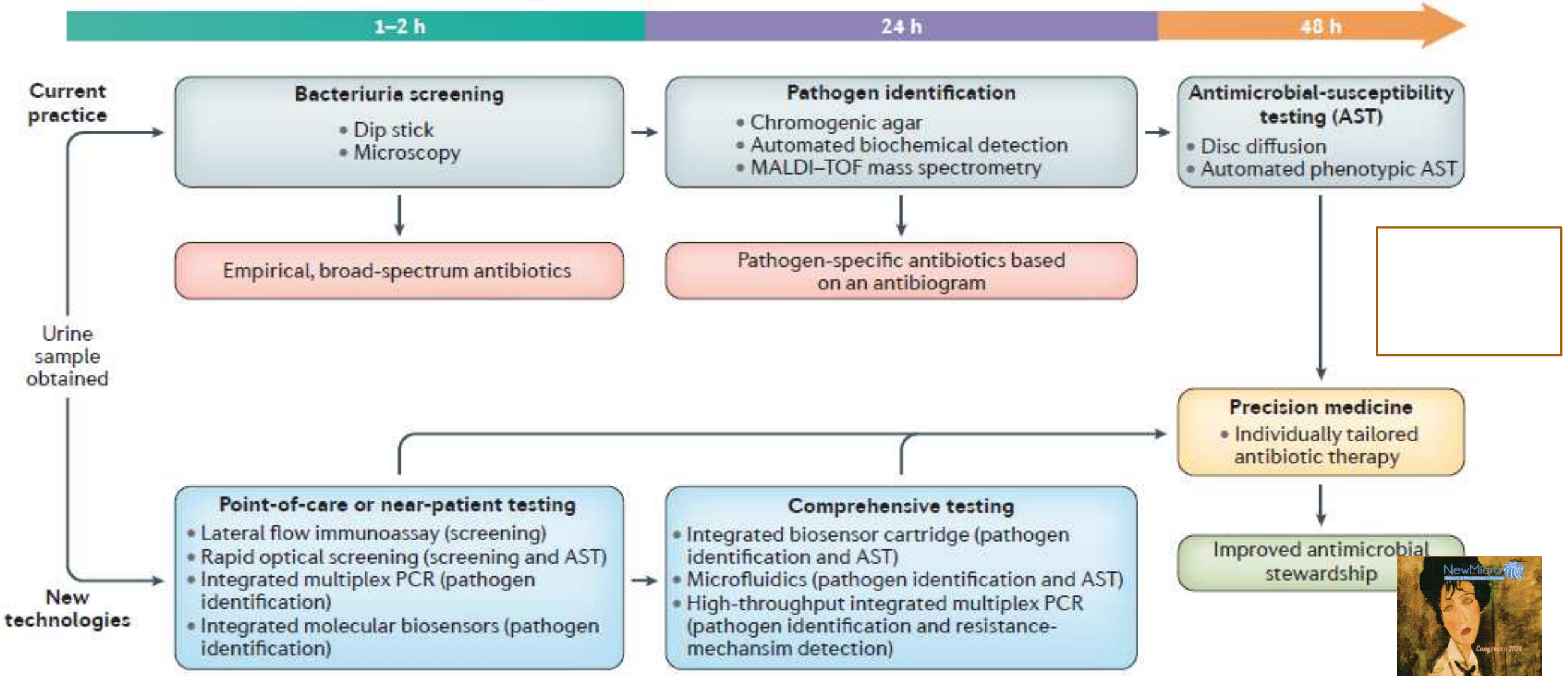
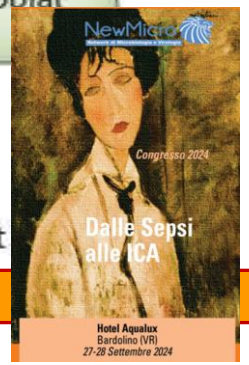


Figure 1 | **Overview of the clinical workflow of existing and future diagnostic technologies for UTI.** In current



New and developing diagnostic technologies for urinary tract infections

Davenport M et al, Narure Reviews - Urology, http://dx.doi.org/10.1038/nrurol.2017.20, 2017

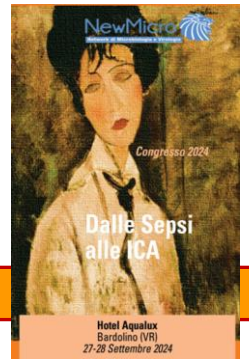
Table 1 | **Approved technologies for pathogen detection**

Technology	Commercial assay	AST	Advantages	Disadvantages	Refs
Nitrite and leukocyte esterase	Dipstick (lateral flow assay)	No	Point of care	Poor specificity	29
Conventional culture	• VITEK • MicroScan	Yes	Standard of care, sensitive, and inexpensive	Time consuming, not translatable to point-of-care applications	25,26
Urinalysis and microscopy	• sediMAx • CLINITEK Atlas • Sysmex UF-1000i • Iris iQ200	No	Fast, detects the presence of bacteria	No pathogen identification	32–34
MALDI-TOF mass spectrometry	• VITEK MS • Bruker MALDI-TOF	Under development	Fast, sensitive, specific, potential for simultaneous AST detection	Expensive for initial equipment	49–54
Fluorescent *in situ* hybridization (FISH)	AdvanDx *Quick*FISH	Under development	Rapid detection, high sensitivity and specificity	Requires multiple probes for all possible urinary pathogens	61–63
Microfluidics	UTI Biosensor Assay (not FDA approved)	Under development	Integrated platform, rapid detection direct-from-patient samples, small footprint	System is not fully automated, poor data from low concentration of bacteria	82,83, 94,95
PCR (clinical isolates)	• GeneXpert • SeptiFast • FilmArray	Resistance-gene probes available	Specific, sensitive, and rapid	Requires multiple probes for all possible urinary pathogens and extensive initial processing	68–73
Immunological-based assays	RapidBac	No	Rapid and inexpensive	Poor specificity and sensitivity	31
Forward light scattering	• Uro-Quick • BacterioScan	Under development	Inexpensive potential for AST	No species indentification	40,41

AST, antimicrobial susceptibility testing; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; MS, mass spectrometry.

Key points

- UTIs are increasingly caused by multidrug-resistant organisms as a result of the overuse of empirical, broad-spectrum antibiotic therapy
- Antimicrobial susceptibility, determined by the phenotypic response to antibiotic exposure, is key for clinical decision making for treating the wide variety of uropathogens and identifying resistance markers
- Existing technologies (such as PCR, fluorescence *in situ* hybridization, and mass spectrometry) and new technologies (such as droplet microfluidic and biosensor platforms) need to focus on direct urine testing to expedite objective diagnoses
- Integrated biosensor–microfluidic platforms have the most potential for point-of-care testing, as they facilitate direct urine analysis and can encompass all assay steps in a compact device
- New technologies are a key step towards improved antimicrobial stewardship

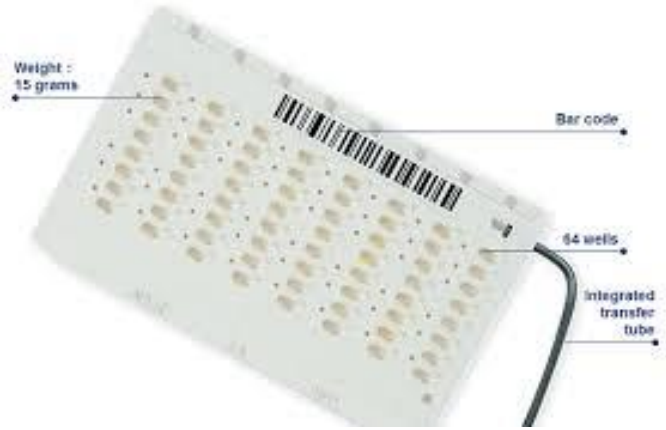


SISTEMI DI IDENTIFICAZIONE MICROBICA E ANTIBIOGRAMMA

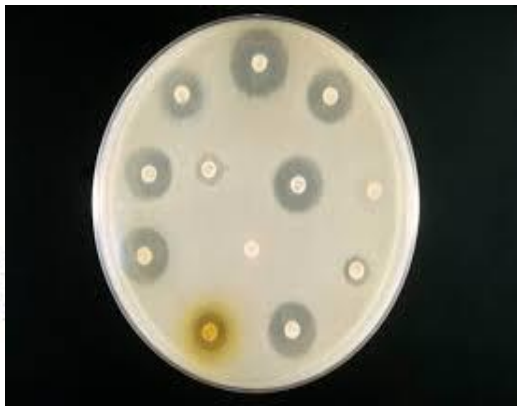
L'antibiogramma è il risultato di un test in cui viene saggiata, *in vitro*, la suscettibilità di un microrganismo espressa in MIC (concentrazione minima inibente) a diversi antibiotici.

La **MIC rappresenta la concentrazione più bassa** (espressa ad es. in µg/mL) di un antibiotico in grado di inibire la crescita di un determinato ceppo batterio.

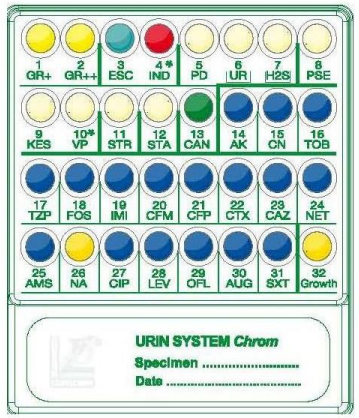
I sistemi automatici



Diffusione su disco



Microdiluzione



Etest

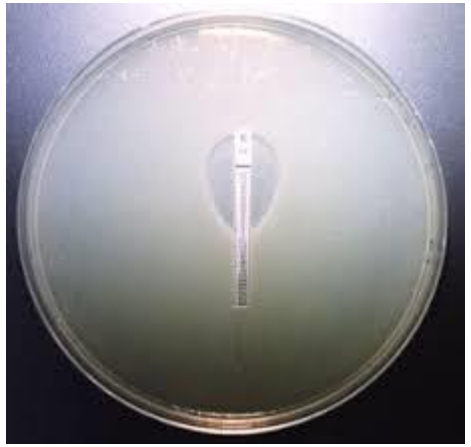


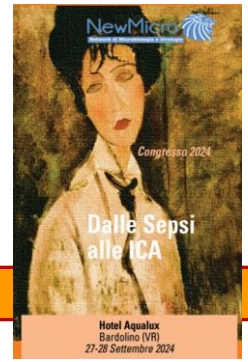
Table 1. Recommendations of the European Association of Urology guidelines [5▪] for the management of ABU

	Screen	Treat	Notes
Healthy patients	☻	☻	
Pregnant women	☺	☺	*Please consult national guidelines*
Postmenopausal women	☻	☻	
Women with recurrent uncomplicated UTI	☻	☻	*The treatment of ABU may be potentially harmful*
Diabetes	☻	☻	
Elderly institutionalized patients	☻	☻	
Patients with renal transplants	☻	☻	
Prior to surgery	☺	☺	*Only in case of urological procedures entering urinary tract and breaching the mucosa*
Patients with indwelling catheters	☻	☻	

Asymptomatic bacteriuria, to screen or not to screen – and when to treat?

Cai T et al, Curr Opin Infect Dis, Vol 27:107, 2017

The table shows the recommendation from the European Association of Urology guidelines in the management of patients with ABU. ABU, asymptomatic bacteriuria; UTI, urinary tract infection.
☺ = recommended.
☻ = not recommended.



Interpretazione dell'esame colturale

Donne adulte

Urinocoltura	Attenzione alle contaminazioni Significativo >=10^5cfu/ml
	Non c'è carica batterica "gold standard" per la diagnosi di UTI
Dipstick test	No evidenza: nitriti pos e/o leucociti pos sono meno predittivi di UTI rispetto alla presenza di segni e sintomi

Gravidanza

Urinocoltura	Gold dtandard
	Eseguire sempre alla prima visita prenatale (A)
	Confermare batteriuria con un secondo campione (A)
	pz con batteriuria confermata da secondo campione va controllata fino al parto (C)
Dipstick test	Non sufficientemente sensibile per lo screening (A)

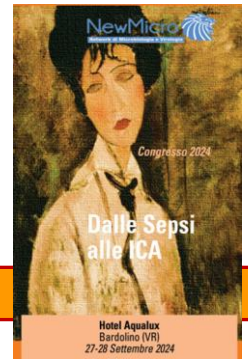
Pz con catetere

Urinocoltura	Attenzione alle contaminazioni Significativo >=10^3cfu/ml
Dipstick test	Non raccomandato

SIGN
Scottish Intercollegiate Guidelines Network

Uomini adulti

Urinocoltura	Significativo >=10^3cfu/ml
Dipstick test	Non raccomandato



Interpretazione dell'esame colturale

La carica batterica clinicamente rilevante

Carica batterica	Tipo di raccolta	Paziente	Patologia	UTI
$>10^3$ cfu/mL	Mitto intermedio	donne	cistiti	acute non complicate
$>10^4$ cfu/mL	Mitto intermedio	donne	pielonefriti	acute non complicate
$>10^5$ cfu/mL	Mitto intermedio	donne		complicate
$>10^4$ cfu/mL	Mitto intermedio	uomini		complicate
$>10^4$ cfu/mL	Catetere	donne		complicate
qualsiasi	Puntura sovrapubica	gravide		
$>10^5$ cfu/mL	2 urinocolture pos per lo stesso germe raccolte a 24h di distanza		Batteriuria asintomatica	

European Association of Urology

Interpretazione dell'esame colturale

European Association of Urology

Bambini

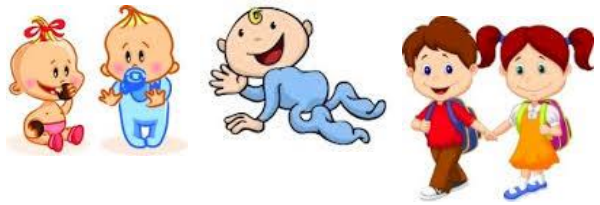
Urinocoltura	Significativo >=10^5cfu/ml

Table 7.2: Criteria for UTI in children

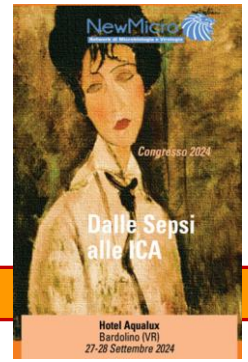
Urine specimen from suprapubic bladder puncture	Urine specimen from bladder catheterisation	Urine specimen from midstream void
Any number of cfu/mL (at least 10 identical colonies)	≥ 1,000-50,000 cfu/mL	≥ 10^4 cfu/mL with symptoms ≥10^5 cfu/mL without symptoms

Urinary tract infection in children
Diagnosis, treatment and long-term management

Issued: August 2007 NICE clinical guideline 54



<3mesi	**Urinocoltura**	Raccomandata
3mesi-3anni	**Urinocoltura**	Raccomandata con e senza sintomi
>3anni	**Urinocoltura**	Raccomandata



Interpretazione dell'esame colturale

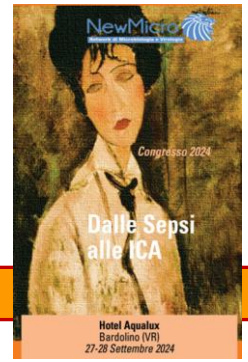
BIMBO
Età 1 anno

Esame chimico fisico

pH	7.0
Densità	1012
Emoglobina	assente
Esterasi leucocitaria	assente
Nitriti	assenti
Emazie	assenti
Leucociti	assenti
Batteri	assenti

Urinocoltura
Positiva per *Escherichia coli*
Carica batterica >1.000.000 UFC/mL

Urinocoltura
Positiva per *Proteus mirabilis*
Carica batterica 1.000.000 UFC/mL



Biosensor diagnosis of urinary tract infections: a path to better treatment?

Mach KE et al, Trends in Pharmacological Sciences, Vol. 32, No. 6, 2011

biosensors in the clinical arena: portability, rapidity, simplicity and cost-effectiveness

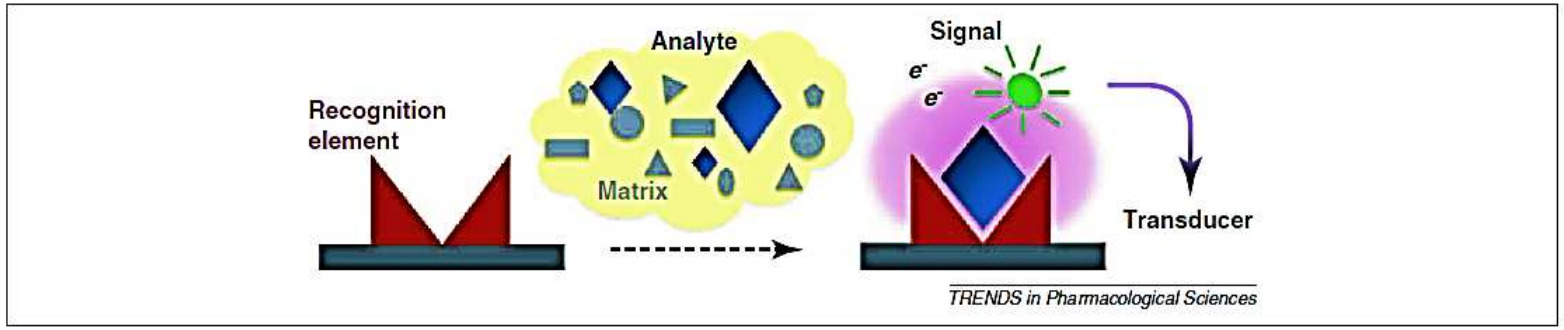


Figure 1. A biosensor is a molecular sensing device composed of a recognition element and transducer. Specific binding of the target analyte to the recognition element generates a measurable signal (e.g. light, electrical current) that is detectable via the transducer [e.g. charge-coupled device (CCD) camera, photodiode, electrode]. Common examples of recognition elements include enzymes, antibodies, and DNA that are able to bind specifically to target analytes, including glucose, ions, protein, and nucleic acids which are indicative of the state of health or disease. The matrix is the biological medium (e.g. blood, urine, saliva) with varying biochemical parameters and nonspecific cells and molecules that could impact upon the performance of the biosensor.

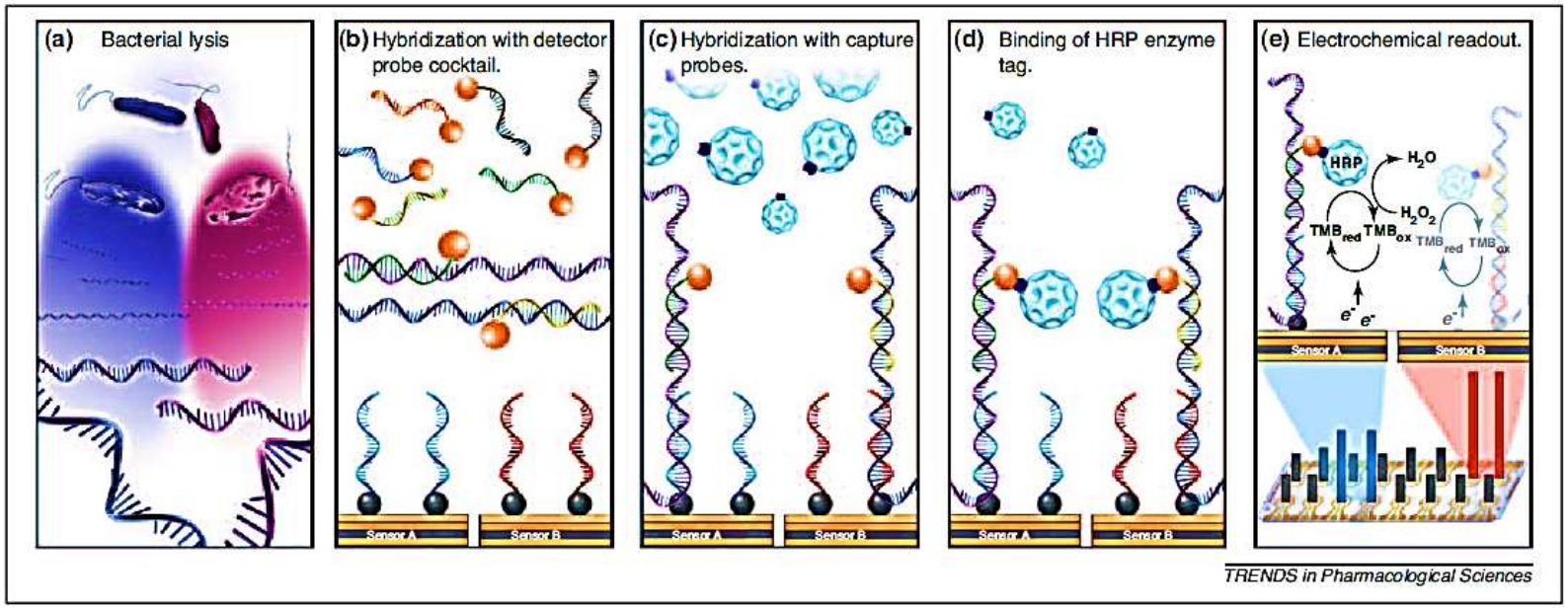
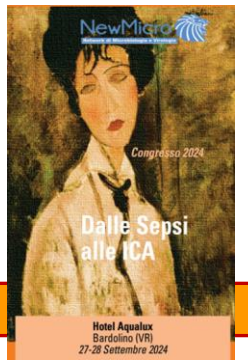


Figure 2. Multiplex pathogen-detection scheme using an array of 16 electrochemical biosensors (UTI Sensor Array). Each sensor is composed of 3 electrodes: working, reference, and counter. (a) Lysis of pathogens in urine samples releases the 16S rRNA target. (b) Hybridization of the 16S rRNA targets with a cocktail of detector probes labeled with fluorescein (orange sphere). (c) Deposition of the 16S rRNA-detector probe hybrid onto the sensor surface (working electrode) for sandwich hybridization with the capture probes. Biotin-labeled (grey sphere) capture probes of different specificities are tethered to the surface of each sensor. (d) Binding of the anti-fluorescein horseradish peroxidase (HRP) enzyme tag to the sandwich hybrid. (e) Oxidation of the HRP substrate H_2O_2 and electron mediator tetramethylbenzidine (TMB) under a fixed voltage generates an electroreduction current. The magnitude of the signal output corresponds to the starting concentration for each pathogen. The limit of detection is 10^4 cfu/ml. Modified from [47] with permission from the copyright holder, Elsevier.

In contrast to blood glucose or urine hCG, detecting the target analytes for UTI require multi-step sample preparation steps, including pipetting (i.e. reagent transfer and mixing), centrifugation (separation and concentration) and washing. Although easily performed in a laboratory setting, they are not practical in POC settings.

If the target analyte is intra-cellular (i.e. nucleic acids), an additional step of bacterial lysis is required.



Biosensor diagnosis of urinary tract infectios: a path to better treatment?

Mach KE et al, Trends in Pharmacological Sciences, Vol. 32, No. 6, 2011

Specifically for UTI, a successful biosensor needs to meet the following criteria: the ability to definitively rule out infection; (ii) the assay needs to be fast, within the POC time-frame to effect treatment planning; (iii) automation of the sample preparation with minimal intervention from the end-user ('plug and play'); (iv) robust assay protocol compatible with urine matrix effect; (v) Incorporation of pathogen identification with antimicrobial susceptibility testing; and (vi) sufficiently versatile to be adaptable for different pathogen profiles in different clinical scenarios.

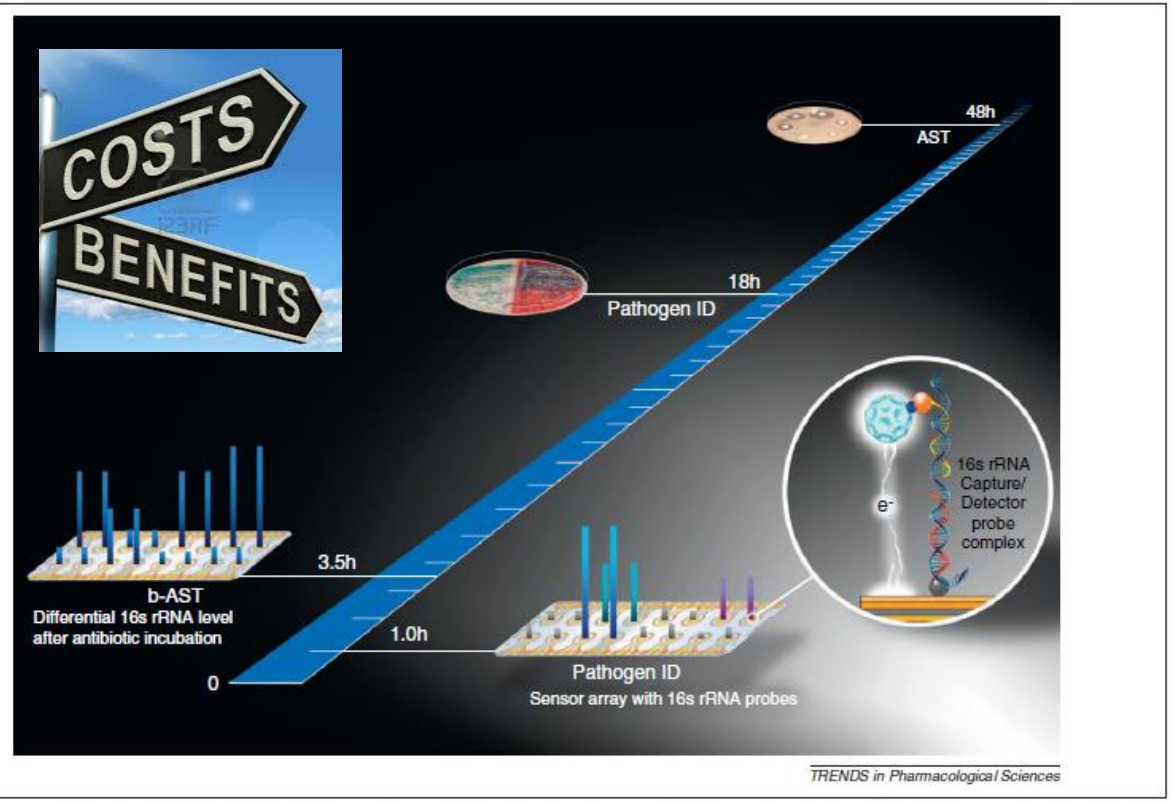
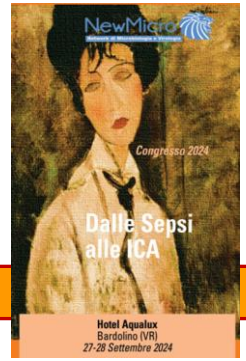


Figure 3. Biosensor diagnosis of UTI is significantly faster than standard culture-based approach. Pathogen identification (ID) and biosensor-based antimicrobial susceptibility test (b-AST) can be completed within 1 and 3.5 h, respectively, compared to 1–3 d for standard culture. Reprinted from [48] with permission from the copyright holder, Elsevier.

Recently we described a biosensor-based AST (b-AST) which combines the versatility of the phenotypic assay with genotypic specificity of the 16S rRNA probes.

Conclusions ... There is a significant need for improved diagnostics, including pathogen identification and antimicrobial susceptibility profiling. Evidence-based treatment plans can therefore be implemented and judicious usage of antibiotics applied. Biosensors offer a promising approach for delivering highly sensitive molecular diagnostic testing in POC settings. With continuing technology advancements and clinical acceptance, they will potentially lead to a paradigm-shift for UTI diagnosis and treatment and serve as a model for other common infectious diseases.



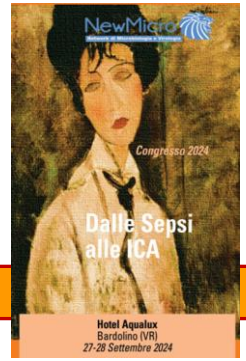
RACCOMANDAZIONI

Il paziente deve essere adeguatamente informato circa le modalità di raccolta del campione

ECMU ed ECU devono essere eseguiti nel minor tempo possibile, o se si suppone invio dopo le 2 h dal prelievo conservare il campione a temp 4-8 °(al fine di ridurre la proliferazione batterica)

Il laboratorio deve valutare idoneità del campione pervenuto per ECMU/ECU (in merito a identìficazione, volume, tempi di trasporto, notificare l anomalia e richiedere nuovo campione)

Livelli diagnostici dell'esame ECU: identificazione di specie e antibiogramma con metodi di microdiluizione, automatici e manuali con identificazione di MIC.



Schistosoma

CREEPY DREADFUL WONDERFUL PARASITES

A PARASITOLOGIST'S VIEW OF THE WORLD

MONDAY, NOVEMBER 12, 2018

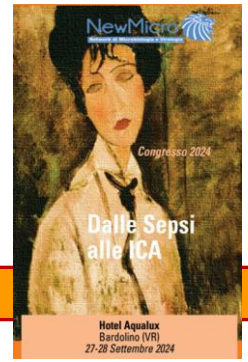
Case of the Week 518

This week's case is a bit of a puzzle for you to put together. The following object was seen in a urine sediment. It was initially moving, but very quickly died. It measures approximately 130 micrometers in length.

Wet prep, 10x objective

Wet prep, 40x objective

Identification? Images are by one of our Clinical Microbiology fellows, Dr. Sarah Jung.



B.Coli + T.vaginalis

Urinary infection due to *Balantioides coli*: a rare accidental zoonotic disease in an addicted and diabetic young female in Iran

S. Soleimanpour and others

JMM Case Reports (2015)

Fig. 1. Trophozoite of *B. coli* with micronucleus, macronucleus and food vacuoles in urine sample. A, macronucleus; B, micronucleus; C, cytostome; D, cilia lining the mouth part, which appeared to be longer than the others (adoral cilia). Magnification, ×1000.

Case presentation: Here, an interesting case of urinary balantiosis in a 35-year-old addicted woman with multiple health problems including spontaneous abortion and diabetes is reported. Her midstream urine sample, collected while all sterile precautions were being taken, demonstrated *B. coli* along with *Trichomonas vaginalis* and bacteria. *B. coli* was identified by its characteristic morphology and rapid rotary motility in the urinary tract, which is an abnormal site for invasion by this parasite.

The patient's fresh midstream urine sample was sent for macroscopic and microscopic examination. Its physical appearance was smoky and turbid, and a urine test strip revealed sugar and proteinuria. Samples were examined

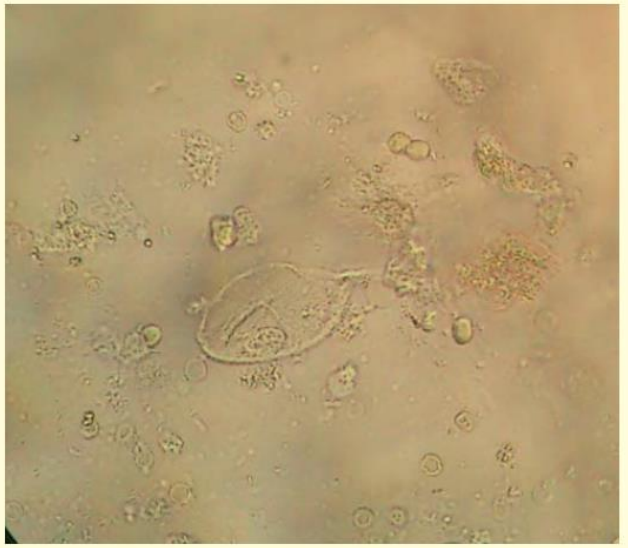
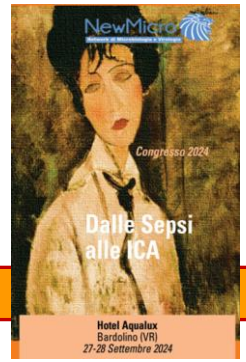


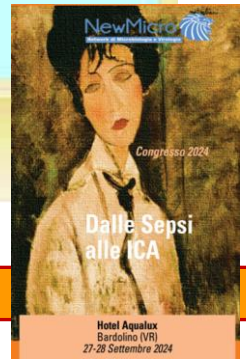
Fig. 2. Trophozoites of *T. vaginalis* in the urine sediment. Magnification, ×1000.



Case Report

Trichomonas vaginalis

Fonte: atlante delle immagini IQ200



CASE REPORT **BK Virus** Roberta Anderlini

Biochimica Clinica 2019; 43(2) e20-e23
DOI: DOI: 10.19186/BC_2019.030

Paziente pediatrico con oliguria e adenopatia cervicale: il ruolo degli analizzatori a cattura di immagine per l'esame standard delle urine

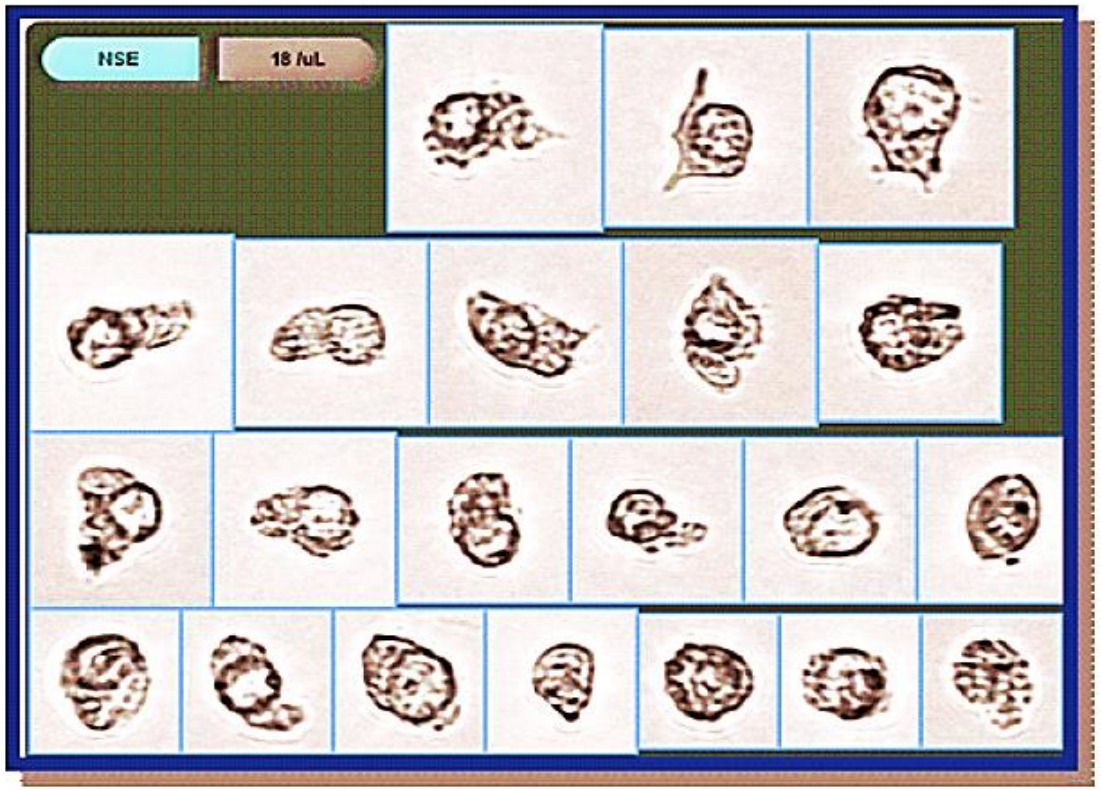
Urinalysis in a pediatric patient with oliguria and cervical lymphadenopathy: role of automated image analysis systems. Automated urinalysis instruments image-based for cell analysis can identify non-squamous epithelial cells (NSE). Among these elements, expert pathologists can distinguish the so called Decoy Cells (DC), Polyomavirus BK (BKV)-infected elements primarily seen in immunocompromised patients. Epstein-Barr virus (EBV) infection can induce a transient immunosuppression in immunocompetent patients, and this could lead to a reactivation of a latent BKV infection in urothelial cells: this is a rare event in pediatric patients. This study reports the case of a 4 year-old child with lateral lymphadenopathy, fever and oliguria. Automated urinary sediment analysis evidenced the presence of many NSEs identifed as DC, generating a subsequent virological investigation with a final diagnosis of concomitant BKV and EBV infection. The combination of an automated technology, an efficient middleware and the expertise of the laboratory professionals , allowed the proper identification of these peculiar reactive elements, which could easily be mistaken for malignant atypical cells.

Sulla base del quadro citologico urinario indicativo di infezione da BKV e della concomitante sintomatologia del bambino suggestiva pe infezione da EBV, si ipotizzava una co-infezione virale d e veniva suggerito il ricorso ad indagini di diagnostica virologica. I risultati confermavano l'infezione primaria da EBV con IgG ed IgM anti-VCA positive (CMIA Architect, Abbott) e IgG anti-EBNA negative (Bio Merieux).

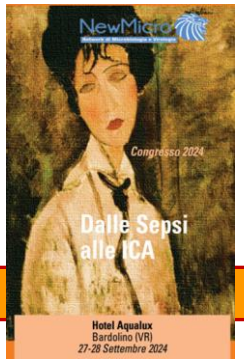
Sul campione urinario venivano ricercati gli acidi nucleici virali mediante PCR Real-Time per la determinazione quali-quantitativa del DNA di BKV (ELITeGroup SPA): il risultato quantitativo di >14.000.000 copie/mL confermava quanto sospettato origonariamente in base al quadro citologico.

L'adeguata identificazione delle cellule in esame ha quindi consentito il corretto trasferimento di informazioni al medico richiedente anche in virtù di questo commento interpretativo al referto: *«Presenza di elementi cellulari di verosimile natura reattiva. Si consiglia esame di controllo previo accordo con il laboratorio».* Dato il decorso autolimitante di questi quadri nel soggetto immunocompetente si è concordato un esame di controllo a distanza, che ha dimostrato , a 2 mesi dalla prima osservazione, la scomparsa degli elementi reattivinel campione.

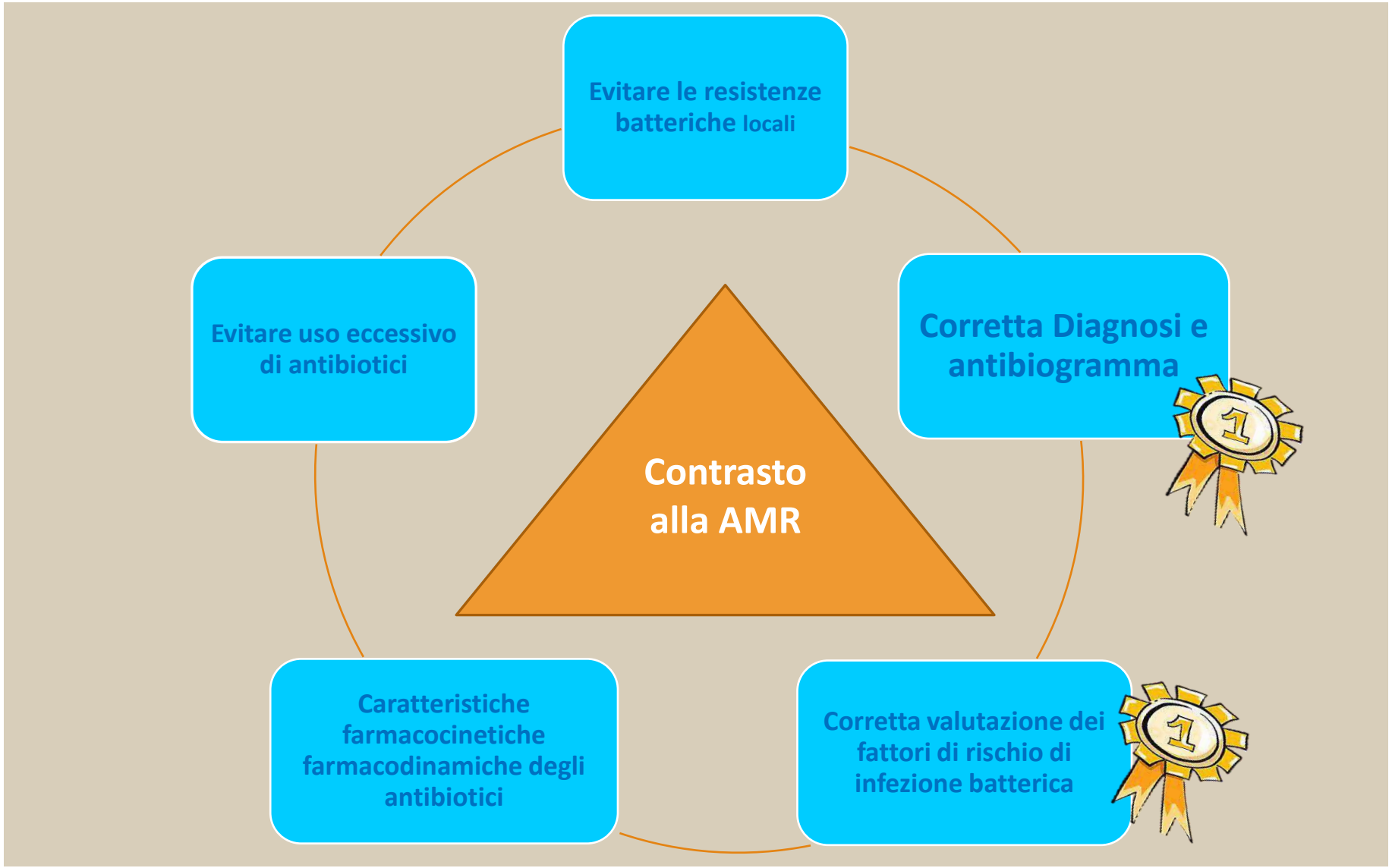
Figura 1
Elementi uroteliali (morfologia tipo decoy cells "comet-like") classificati nella categoria cellule epiteliali non squamose: si tratta di cellule isolate con citoplasma a margine irregolare, ipertrofia nucleare e addensamenti cromatinici (elementi 18/µl). Analizzatore IRIS iQ200 Sprint



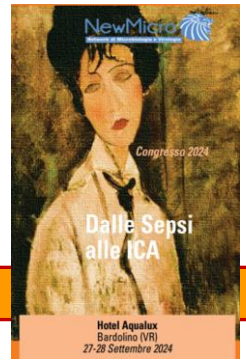
In conclusione, in un laboratorio ad elevata produttività, gli analizzatori urinari a microscopia automatizzata basati su tecnologia a rete neurale associati a regole personalizzate di validazione e revisione dei campioni, un efficiente middleware e l'esperienza degli operatori, hanno consentito la corretta identificazione e segnalazione di cellule altrimenti confondibili con elementi atipici/maligni che avrebbero indotto ad approfondimenti diagnostici inutili e potenzialmente dannosi.



RACCOMANDAZIONI PER EVITARE INSORGENZA RESISTENZE BATTERICHE



EAU Guidelines on urological infections 2020



Take home message

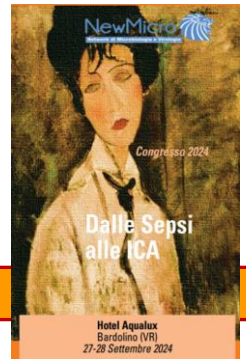
Appropriatezza prescrittiva, obbligo **indicazione diagnostica**, corretta fase **preanalitica**

Standardizzazione procedure analitiche per IVU **con identificazione di specie e antibiogramma (MIC)**

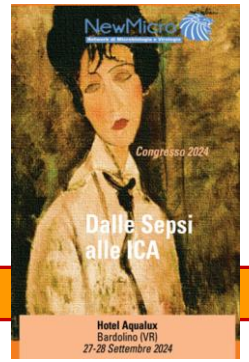
Programmi di Sorveglianza delle IVU Programma AR-ISS

Terapia mirata con antibiotici – Riduzione MDR

Programma ONE HEALTH



Conclusioni

- Dare corrette indicazioni agli utenti sulle modalità di raccolta, conservazione e trasporto del campione di urine
- Rapporto clinici-microbiologi
- Creare profili analitici diversi in base al tipo di campione raccolto
- Valutare età, sesso e condizioni cliniche prima della refertazione dell'esame colturale
- Confrontare esito urinocoltura (germe vs cfu/ml) con esiti precedenti
- Referto ad hoc: utilizzare commenti che invitino il clinico a valutare con maggiore attenzione la correlazione tra il risultato microbiologico e il contesto clinico, per evitare trattamenti inappropriati
- Mettere in atto tutte le strategie per non refertare antibiogrammi relativi a isolamenti che sono espressione di contaminazione/ colonizzazione.

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