MINIREVIEW

# Asymptomatic bacteriuria: prevalence rates of causal microorganisms, etiology of infection in different patient populations, and recent advances in molecular detection

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## Abstract

Bacteriuria, or the presence of bacteria in urine, is associated with both asymptomatic and symptomatic urinary tract infection and underpins much of the dynamic of microbial colonization of the urinary tract. The prevalence of bacteriuria in dissimilar patient groups such as healthy adults, institutionalized elderly, pregnant women, and immune-compromised patients varies widely. In addition, assessing the importance of 'significant bacteriuria' in infected individuals represents a diagnostic challenge, partly due to various causal microorganisms, and requires careful consideration of the distinct etiologies of bacteriuria in different populations and circumstances. Recent molecular discoveries have revealed how some bacterial traits can enable organisms to grow in human urine, which, as a fitness adaptation, is likely to influence the progression of bacteriuria in some individuals. In this review, we comprehensively analyze currently available data on the prevalence of causal organisms with a focus on asymptomatic bacteriuria in dissimilar populations. We evaluate recent advances in the molecular detection of bacteriuria from a diagnostic viewpoint and briefly discuss the potential benefits and some of the challenges of these approaches. Overall, this review provides an update on the comparative prevalence and etiology of bacteriuria from both microbiological and clinical perspectives.

## Introduction: bacteriuria in asymptomatic and symptomatic urinary tract infection

The term 'asymptomatic bacteriuria' (ABU or ASB) is effectively synonymous with asymptomatic urinary tract infection (UTI) in defining the isolation of a specified semi-quantitative count of bacteria in an appropriately collected urine specimen from a person without signs or symptoms related to UTI (Rubin *et al.*, 1992; Nicolle *et al.*, 2005). The semi-quantitative count used for defining 'significant bacteriuria' is a matter of debate and will be discussed in the section on diagnostics. Bacteriuria is not restricted to ABU; it is also used to characterize symptomatic UTI (sUTI) based on clinical presentation and semi-quantitative counts as a diagnostic marker for grading infection. In this review, we discuss bacteriuria related to ABU and how it affects different patient populations. We review the diversity of causal microorganisms and how distinct etiologies of bacteriuria occur in dissimilar populations. Finally, we summarize recent advances in molecular detection approaches for the diagnostic assessment of bacteriuria.

Establishment of ABU in an individual depends on the entry of an organism with bacteriuric potential into the urinary tract. Long-term ABU is defined as a persistent infection of > 3 weeks (to several years) of duration and stems from an organism being sufficiently adapted to urine to survive host defenses. Bacteriuria can originate from the bladder or the kidneys. Differentiating bladder

vs. renal origin bacteriuria is challenging but important from an etiological viewpoint because of the high incidence of renal infection in some patient populations such as community-dwelling elderly adults (Patterson & Andriole, 1997). The potential for bacteriuria to be both an infection and a risk factor for subsequent development of sUTI highlights the complex nature of the condition (Hooton *et al.*, 2000; Geerlings *et al.*, 2001). This helps to reconcile the contradictory roles of ABU in protecting some individuals against sUTI (Hull *et al.*, 2000; Wullt, 2003; Sunden *et al.*, 2006), but predisposing others to developing sUTI. Clinically, treatment is not recommended as routine practice for ABU, excepting for pregnant women and individuals undergoing invasive genitourinary procedures. This is because of adverse events and a lack of efficacy in preventing subsequent sUTI (Nicolle, 2006). Instead, ABU is managed according to the clinical situation (Johansen *et al.*, 2011). A key concept is that a single definition does not sufficiently describe ABU from an etiological standpoint, which mirrors emerging concepts of imperfect definitions for complicated and uncomplicated sUTI (Johansen *et al.*, 2011). Hence, bacteriuria comprises an etiological spectrum that encompasses diverse causal bacteria with distinct prevalence rates in different patient groups and involves different host factors that predispose to infection.

## Prevalence of ABU causal microorganisms in distinct patient populations

Multiple species from fourteen genera cause essentially all culture-detectable ABU. Noncultivable bacteria were recently described (Wolfe *et al.*, 2012) and form part of the urine microbiome in healthy adults and those with neuropathic bladder (Siddiqui *et al.*, 2011; Fouts *et al.*, 2012). Whether noncultivable microorganisms influence bacteriuria due to cultivable organisms is unknown. *Escherichia coli* causes most ABU. Other Enterobacteriaceae (*Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter* spp., *Providencia stuartii*, and *Morganella morganii*), nonfermentative Gram-negative bacilli (e.g. *Pseudomonas aeruginosa*), and Gram-positive bacteria including *Enterococcus* spp., *Staphylococcus aureus,* and coagulase-negative staphylococci (mainly *Staphylococcus saprophyticus*) also cause ABU (Ronald, 2002, 2003; Nicolle *et al.*, 2005) (Table 1). ABU due to *Streptococcus agalactiae* is relatively common in pregnancy, particularly in the form of low-count bacteriuria, and has been associated with adverse obstetric outcomes (Le *et al.*, 2004; Anderson *et al.*, 2007; Kessous *et al.*, 2012). This organism has also been a focus of several bacteriuria studies in nonpregnant adults (Hernaiz *et al.*, 2004; Ulett *et al.*, 2009; Tan *et al.*, 2012). All of these species can also cause acute uncomplicated UTI (symptomatic bladder infection with frequency, urgency, dysuria, and/or suprapubic pain in a person with normal genitourinary tract function) (Hooton & Stamm, 1997; Nicolle *et al.*, 2005; Johansen *et al.*, 2011).

The prevalence of ABU differs dramatically in distinct patient populations and is influenced by gender, age, medical interventions, and comorbidities. Overall prevalence rates are summarized in Table 1, which illustrates how causal organisms disproportionately affect different patient populations. For example, *E. coli* is less prevalent among healthy men and patients with indwelling catheters; enterococci have been cultured from almost a quarter of bacteriuric healthy men, but only 3–4% of bacteriuric pregnant women. Staphylococci rarely cause ABU in healthy adults, but are relatively prevalent among ABU pregnant women, diabetic patients, and community-dwelling elderly men. Detailed prevalence data are shown with supporting references in Supporting Information, Table S1. Why different bacteria disproportionately affect different patient populations is largely unknown. There are, however, unique frequencies of host characteristics in sUTI as defined by the causal organism (Tabibian *et al.*, 2008), implying that host environment is important. Next, we will compare several etiologies of ABU: in pregnancy, diabetes, elderly patients, and controversies regarding women with urge incontinence.

## Etiology of ABU in pregnancy, diabetes, and elderly individuals

ABU in pregnancy relates to anatomic and physiologic changes in the urinary tract that alter the host environment. Compression of the ureters at the pelvic brim may predispose to upwards reflux of urine; thus, ABU more readily progresses to pyelonephritis during pregnancy. Decreased concentration of urine, glucosuria, and progesterone effects (promote ureteric dilatation) also influence infection (Patterson & Andriole, 1997). ABU prevalence rates in pregnancy range between 1.9% and 15% (Table S1). The risk of vertical transmission of bacteria is important, particularly given ABU is more persistent and more frequently progresses to sUTI in pregnancy compared with other populations (e.g. 20–40% of untreated cases progress to sUTI, which is complicated by premature delivery in 20–50%) (Patterson & Andriole, 1997; MacLean, 2001). This may relate to lower antibacterial activity of urine during pregnancy (Patterson & Andriole, 1997). Routine culture-based screening at 12–16 weeks remains the consensus recommendation, and despite evaluation of various screening tools (Patterson & Andriole, 1997; Deville *et al.*, 2004; Schnarr & Smaill, 2008;

**Table 1.** ABU prevalence in distinct patient populations (% below patient group) and comparative peak prevalence rates of causal bacteria in studies to date

| Causal organism* | Healthy adult women (1–9%) | Healthy adult men (1–2%) | Pregnant women (2–15%) | Diabetic adults (1–30%) | Community dwelling elderly (2–50%) | Institutionalized adults/elderly (14–75%) | Patients with indwelling catheter (9–100%) |
|---|---|---|---|---|---|---|---|
| High | | | | | | | |
| *Escherichia coli* | 91 | 25 | 86 | 80 | 80 | 60 | 35 |
| *Enterococcus* spp. | 33 | 23 | 4 | 13 | 8 | 10 | 25 |
| *Staphylococcus* spp. | 2 | 7 | 24 | 16 | 53 | 10 | 6 |
| *Proteus* spp. | | | 9 | 35 | 10 | 24 | |
| Moderate | | | | | | | |
| *Klebsiella pneumoniae* | 6 | 8 | 16 | 28 | 17 | 22 | 21 |
| *S. agalactiae* | 6 | 5 | 26 | 12 | 10 | | 4 |
| *Pseudomonas* spp. | | 12 | 6 | | 18 | 14 | 25 |
| *Enterobacter* spp. | | | 3 | 7 | | | 21 |
| Low | | | | | | | |
| *Providencia* spp. | | | 6 | | 2 | 16 | |
| *Gardnerella vaginalis* | | | 15 | 4 | | | |
| *U. ureolyticum* | | | 15 | | | | |
| Rare | | | | | | | |
| *Morganella morganii* | | | | | 2 | | 10 |
| *Serratia* spp. | 5 | 2 | | | | | |
| *Citrobacter freundii* | | | | 4 | 2 | | |

*For causal organisms, peak prevalence is the highest reported prevalence rate from published studies; all % are rounded; references used are provided in Table S1 that also presents % range, gender and age analyses where appropriate, alongside specific references; causal microbes grouped according to highest peak prevalence rate in at least one patient population [High (> 30%), Moderate (> 20%), Low (> 10%) and Rare (≤10%)]. Shading key: >80% of cases; 30–80%; 20–30%; 10–20%; 5–10%; 2–5%; N/A

Lumbiganon *et al.*, 2010), the most effective approaches to screening and treatment continue to be reassessed (Guinto *et al.*, 2010; U.S. Preventive Services Task Force U, 2010). *Escherichia coli* causes up to 86% of cases; however, this varies widely; *S. agalactiae* is also important and was reported in 26% of cases in diabetes gravidas in one study (Table S1). *Streptococcus agalactiae* bacteriuria of any count in pregnancy necessitates antibiotic treatment. The benefits of treatment at low counts, however, remain uncertain (Allen *et al.*, 2012). For other organisms, counts $\geq 10^5$ CFU $mL^{-1}$ are treated according to the causal bacteria (Le *et al.*, 2004; Schnarr & Smaill, 2008; Cormican *et al.*, 2011; Allen *et al.*, 2012). Screening and treatment reduce the risk of pyelonephritis and adverse obstetrical outcomes; however, the optimal duration of treatment is yet to be defined (Patterson & Andriole, 1997; Smaill, 2001; Lin & Fajardo, 2008; Schnarr & Smaill, 2008).

The etiology of ABU in diabetes is interesting because ABU occurs three times more often in diabetic women compared with nondiabetic women, but no such association is found in men (Ronald & Ludwig, 2001; Dalal *et al.*, 2009). Risk factors include the duration of diabetes, degree of metabolic control, diabetic complications including neuropathy, and previous UTI (Table S1) (Dalal *et al.*, 2009; Papazafiropoulou *et al.*, 2010). *Escherichia coli* is most frequently isolated, but *Klebsiella* and *Proteus* are also common. ABU in diabetes is difficult to eradicate, and up to 20% of untreated women remain bacteriuric with the original organism long term (Dalal *et al.*, 2009). In women with type 2 diabetes, ABU is associated with a higher incidence of sUTI and urosepsis; however, studies have not shown any increased incidence of renal impairment long term (Geerlings *et al.*, 2001). While sUTI follows a more complicated course in diabetic patients, screening for ABU is not recommended because treatment does not reduce the incidence of sUTI or pyelonephritis (Ooi *et al.*, 2004). The impact of therapeutic inhibitors of sodium–glucose transport proteins (that mediate 90% of glucose reabsorption in kidneys), such as canaglifozin, is unclear. Such drugs increase urinary glucose excretion, which was associated with increased sUTI in one study (Ferrannini *et al.*, 2010); however, there was no association with increased ABU or sUTI in a randomized trial (Nicolle *et al.*, 2012). Longer-term studies are needed to determine whether increased glucose availability (for microorganisms) in urine might contribute to progressive bacteriuria.

The etiology of ABU in elderly adults is multifactorial; risk factors include anatomic abnormalities (e.g. prostate

obstruction), hormonal and metabolic changes (e.g. estrogen decrease, diabetes), neurologic disorders, and poor peri-anal hygiene (Wagenlehner *et al.*, 2005). ABU is especially prevalent among residents of long-term care facilities; up to 75% of institutionalized women and 52% of men have been shown to be bacteriuric (Table S1). This contrasts to lower prevalence rates among community-dwelling elderly adults. Among non-*E. coli* causal organisms, *P. mirabilis*, *Klebsiella*, *Pseudomonas,* and staphylococci are prevalent (Table S1). ABU in the elderly is not associated with either increased morbidity or mortality. Because treatment does not improve outcomes but frequently leads to reinfection with resistant organisms, neither screening nor treatment is recommended (Abrutyn *et al.*, 1994; Nicolle *et al.*, 2005; Nicolle, 2006). In addition, distinguishing ABU from sUTI is difficult because elderly patients have a smaller bladder capacity (thus often have frequency of micturition). Elderly patients also often have reduced fluid intake, which makes their urine concentrated and somewhat malodorous. Other atypical presentations are common, in that elderly patients with bacteriuria may simply appear unwell and confused; there is also a high prevalence of pyuria in asymptomatic individuals, which can complicate urinalysis interpretation (van Duin, 2012).

## Current controversy: etiology of bacteriuria in women with urge incontinence

The etiology of bacteriuria in women with urge incontinence (i.e. overactive bladder syndrome; OAB) is a current matter of debate. These individuals generally complain of frequency and urgency of micturition; suprapubic pain may be noted if the spasms of the detrusor muscle are intense. If such patients are found to have two cultures of the same species 24 h apart, with bacteriuria $\geq 10^5$ CFU $mL^{-1}$ of uropathogens (Grabe *et al.*, 2009), in the absence of dysuria, controversy now exists as to whether such women should be considered as having ABU, or in fact, a sUTI that warrants treatment. Recent evidence indicates that 26% of women with severe OAB may have $\geq 10^5$ CFU $mL^{-1}$ without the classical features of dysuria or malodorous urine, compared with 4% of age-matched continent controls (Walsh *et al.*, 2011). The role of urine leakage onto the perineum with possible facilitation of bacterial colonization of the urethral orifice remains controversial also, because patients with OAB leak urine intermittently, not continuously. Recent data regarding the treatment of bacteriuria in OAB showed significant benefit for urgency and frequency of micturition (Gill *et al.*, 2011a, b), but a randomized controlled trial is currently pending.

## What factors influence the progression of bacteriuria?

Recent reviews on the pathogenesis of sUTI (Nielubowicz & Mobley, 2010; Hannan *et al.*, 2012; Ulett *et al.*, 2013) enable us to focus here on the progression of bacteriuria specifically. Whether short-term bacteriuria progresses to long-term ABU depends on both host and microorganism traits. Overall, < 1% of healthy adults with ABU will progress to long-term bacteriuria, and most will not harbor the same strain of causal organism over time (Hooton *et al.*, 2000). Thus, turnover of causal organisms is dynamic, and colonizing strains are often replaced by other strains during infection. For *E. coli*, long-term bacteriuria was recently shown to select for attenuated virulence phenotypes (Salvador *et al.*, 2012). Most microorganisms have low bacteriuric potential (i.e. cannot survive/grow in urine), and urine has natural antimicrobial properties, which probably impacts on strain replacement. The potential contribution of bacterial growth in urine to progression of ABU has been highlighted by several probiotic approaches aimed at reducing the incidence of sUTI (Hull *et al.*, 2000; Wullt, 2003; Sunden *et al.*, 2006; Watts *et al.*, 2012).

Host characteristics also influence the progression of bacteriuria. Clinically, for example, this is evidenced by the increased incidence of infection in asymptomatic elderly adults, which is disproportionally associated with residents of long-term care facilities (van Duin, 2012). In part, these differences reflect the role of comorbidities such as neurologic impairment in predisposing individuals to ABU (Table S1). Key differences in innate immune responses between individuals and genetic background are also important. For example, TLR4 promoter variants that are linked to reduced expression of TLR4 and reduced innate immune responses are associated with ABU, as recently reviewed (Ulett *et al.*, 2013). An evaluation of treatment data also offers some insight into the role of the host. In contrast to sUTI, which is resolved in most cases by treatment (Hooton, 2003; Gupta *et al.*, 2011), therapy is not routine for ABU (Nicolle *et al.*, 2005; Nicolle, 2006). While treatment can halt the progression of bacteriuria and reduce the incidence of infection (Schneeberger *et al.*, 2012), patients infected with *E. coli* experience recolonization with the same or similar organism at surprisingly high rates (Dalal *et al.*, 2009). These data reveal the difficulty in achieving bacteriological cure for ABU and imply that bacteriuria involves frequent recolonization of the host. This is consistent with the view that ABU in adult women is seldom permanently eradicable (Nicolle *et al.*, 2005). Thus, influences of both the microorganism (survival/growth in urine, strain replacement) and host (comorbidities, urine

antimicrobial activity, recurrent infection, therapy) govern the progression of bacteriuria in infected individuals.

## Advances in diagnostics: emerging molecular detection methods

Detection of bacteriuria in the diagnostic laboratory continues to rely on culture-based approaches, which alone have inadequate sensitivity and specificity to define 'significant' bacteriuria in diverse clinical scenarios (Wilson & Gaido, 2004). Indeed, there is no fixed number of significant bacteriuria that can be applied to all forms of UTI (including ABU) under all circumstances (Grabe *et al.*, 2009). The threshold of 'significance' for clean catch specimens was historically $> 10^5$ CFU mL$^{-1}$, as reviewed elsewhere (Rubin *et al.*, 1992; Wilson & Gaido, 2004); however, the importance of the clinical scenario has resulted in lower thresholds of $> 10^2$, $> 10^3$, and $> 10^4$ CFU mL$^{-1}$ for different conditions (Lipsky *et al.*, 1987; Le *et al.*, 2004; Wilson & Gaido, 2004; Johansen *et al.*, 2011). For example, the diagnostic cutoff for sUTI/ABU of $10^5$ CFU mL$^{-1}$ for patients with OAB has been lowered to $10^3$ CFU mL$^{-1}$, as recommended by the European Association of Urology for Women (Grabe *et al.*, 2009). Lower thresholds improve sensitivity without undue impracticality, although have not yet translated widely into clinical practice (Walsh & Moore, 2011). The importance of low counts for sUTI is discussed elsewhere (Kunin *et al.*, 1993; Patterson & Andriole, 1997); but for ABU, the importance of low counts remains uncertain (Stamm & Hooton, 1993; Patterson & Andriole, 1997). In healthy adult men, ABU is typically defined by a single voided specimen with one species $\geq 10^5$ CFU mL$^{-1}$; in healthy adult women, two consecutive voided specimens with the same species $\geq 10^5$ CFU mL$^{-1}$ are used. In both groups, a single catheterized specimen with a species $\geq 10^2$ CFU mL$^{-1}$ defines ABU (Nicolle *et al.*, 2005). Problems of culture-based methods include low-count organisms coexisting with dominant organisms that tend to be missed by routine culture (Sakai, 1995). Culture-based screening approaches also often fail to identify women who subsequently develop sUTI (Patterson & Andriole, 1997). Costs are also high given that urine samples are among the most numerous of specimen types sent for microbiology studies (US$2.20–2.45 per sample for agar/spot test reagents, excluding labor) (D'Souza *et al.*, 2004). Other limitations of culture-based methods are accentuated in some patient populations. For example, atypical presentations in elderly adults who exhibit unusual symptoms of UTI (or none at all) make interpretation difficult (Barkham *et al.*, 1996; Gau *et al.*, 2009; Woodford *et al.*, 2011). Culture-based methods have been reported to miss up to half of ABU cases in pregnant women (McIsaac *et al.*, 2005), and reports of 'no growth' in symptomatic patients continue to be problematic (Hooton, 2012).

Screening for prognostic markers that differentiate between ABU and sUTI supports the interpretation of culture results (Graham & Galloway, 2001). Leukocyte esterase and pyuria as a marker of sUTI have been used for many years but are less useful in diabetic and elderly individuals. Dipstick analysis (for nitrites and esterase) and direct microscopy have poor positive and negative predictive values for ABU and are of limited value in distinguishing sUTI (Hooton & Stamm, 1997; Lin & Brown, 2010). Summaries of the sensitivities and specificities of some of these tests are reviewed elsewhere (Le *et al.*, 2004). Lactoferrin, proteinuria, microalbuminuria, and volatile organic compounds have also been investigated to support culture results (Arao *et al.*, 1999; Aathithan *et al.*, 2001; Nicolle, 2007). Urinary interleukin-6 and interleukin-8 levels have shown some potential prognostic value for differentiating sUTI, especially in children, as reviewed elsewhere (Nanda & Juthani-Mehta, 2009). Recently, a commercial system based on automated microscopy that analyzes cells and particles consistent with bacteria was shown to decrease the negative culture rate by > 50%, but was reliant on operator input (Falbo *et al.*, 2012). Another recently reported flow cytometry–based system achieved similar sensitivity and specificity and was able to reduce the number of urine cultures by 43% (Pieretti *et al.*, 2010). These data were compared with a high rate of false negatives as reported by another group, which would preclude this method as a routine screening approach to exclude urine samples from culture (Brilha *et al.*, 2010). Overall, however, most flow cytometry–based studies reported so far have found few false positives and negatives, implying good potential for clinical application (Okada *et al.*, 2000; Jolkkonen *et al.*, 2010; Broeren *et al.*, 2011; Gutierrez-Fernandez *et al.*, 2012).

Aside from flow cytometry–based methods, new molecular detection methods that could reduce the need for urine culture and ease associated costs and workload are being developed. These are summarized in Fig. 1. Several molecular detection methods are based on the detection of bacterial 16S rRNA genes with dsDNA probes and molecular beacons with target sequences homologous to pan-prokaryotic rRNA or specific microorganisms. Using 16S rRNA gene is attractive because of relative target stability and high copy number per bacterial cell. The use of fluorescent dsDNA probes achieved good sensitivity for low level bacteriuria in one study (Riahi *et al.*, 2011). However, like other molecular methods described to date, no clinical evaluation has been reported. Another promising method that was able to identify most uropathogens from urine is a system using 16S rRNA genes captured

on gold film electrodes (Liao *et al.*, 2006, 2007). In this capture oligonucleotide method, universal bacterial 16S rRNA genes mixed with species- or group-specific labeled probes identified more than 90% of uropathogens at very low levels within 40 min. Addition of lactoferrin detection to this system could enable the evaluation of pyuria. The labor and costs of such a system would be higher than culture, but would eventually be offset with automa-

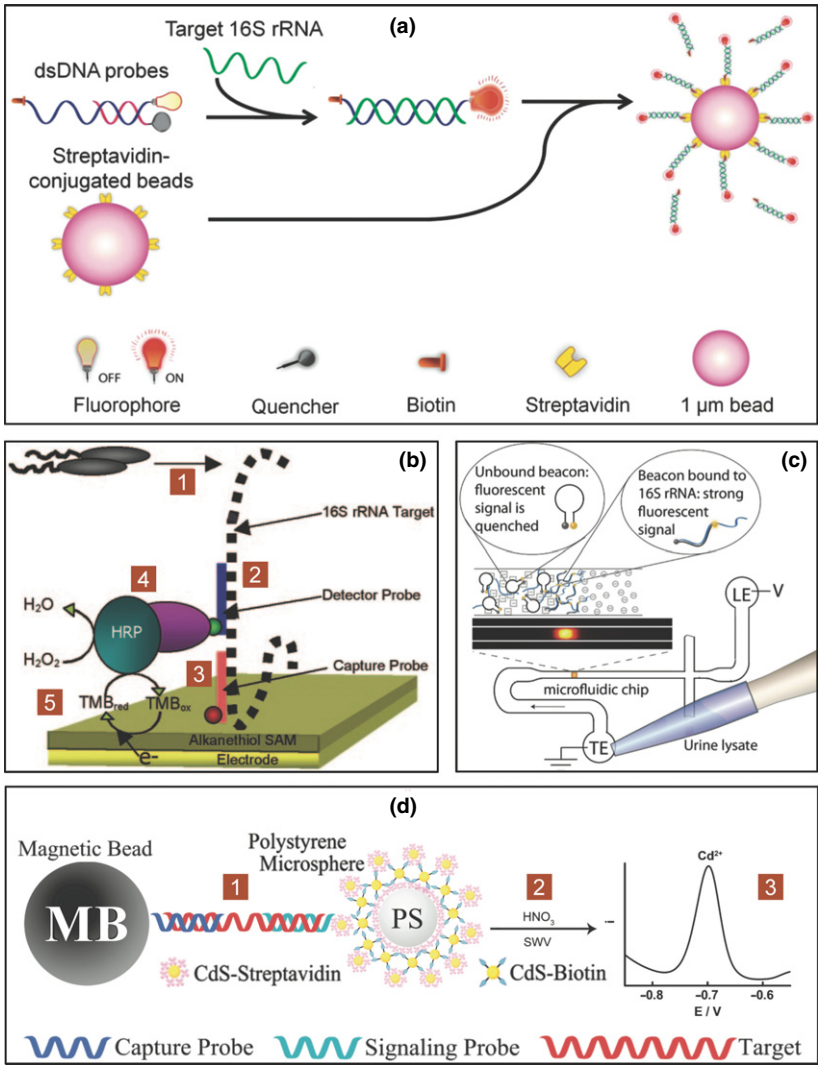


**Fig. 1.** Emerging molecular detection methods for bacteriuria. (a) Microparticle-conjugated dsDNA probes for microorganism-specific nucleic acid. When in the presence of the target, a fluorophore probe is thermodynamically driven to hybridize to the target, which replaces a quencher probe. Probes are captured by streptavidin-coated microparticles (Riahi *et al.*, 2011). (b) Electrochemical sensors illustrating (1) bacteria lysis to release 16S rRNA gene target (dashed line), (2) hybridization of target with fluorescein (green circle)-labeled detection probe (blue), (3) hybridization of target with biotin (red circle)-labeled capture probe (orange), (4) binding of antifluorescein antibody HRP conjugate to target-probe sandwich, and (5) generation of current by transfer of electrons to the electron transfer mediator, TMB (Liao *et al.*, 2006). (c) Molecular beacons to detect microorganism-specific 16S rRNA genes. Similar to microparticle conjugates, these use spatial separation of fluorophore and quencher to enable a strong and sequence-specific increase in fluorescence in the presence of the target. Fluorescing molecular beacons hybridized to synthetic oligonucleotides are shown as a bright orange spot on a black background (Bercovici *et al.*, 2011). (d) Quantum dot technology using cadmium detection, illustrating (1) sandwich complexes through dual hybridization, (2) dissolution of the assembled CdS–streptavidin quantum dots with nitric acid ($HNO_3$), and (3) voltage monitoring of released $Cd^{2+}$ (Xiang *et al.*, 2011). *All panels reprinted with written permission: Riahi R, Mach KE, Mohan R, Liao JC & Wong PK, Analytical Chemistry 83: 6349–6354, Copyright (2011) American Chemical Society, Liao JC, Mastali M, Gau V, et al., Journal of Clinical Microbiology 44: 561–570, Copyright (2006) American Society for Microbiology, Bercovici M, Kaigala GV, Mach KE, Han CM, Liao JC & Santiago JG, Analytical Chemistry 83: 4110–4117, Copyright (2011) American Chemical Society, and Xiang Y, Zhang H, Jiang B, Chai Y & Yuan R, Analytical Chemistry 83: 4302–4306, Copyright (2011) American Chemical Society.*

tion and volume manufacturing (Liao *et al.*, 2006, 2007; Mohan *et al.*, 2011; Patel *et al.*, 2011), as noted for microfluidics (Mariella, 2008). Molecular beacons combined with 16S rRNA genes in a microfluidic chip in another study, using 'isotachophoresis technology', could analyze samples within only 15 min but were limited by a sensitivity of $10^6$ CFU $mL^{-1}$ (Bercovici *et al.*, 2011). For this approach, further improvements in sensitivity would likely enable coverage of other clinically relevant bacteriuria ranges between $10^3$ and $10^5$ CFU $mL^{-1}$.

An alternative molecular method to quantify bacteriuria without culture utilizes quantum dot technology based on a cadmium ion electronic detection system (Xiang *et al.*, 2011). This system uses polystyrene beads covalently coated with CdS-streptavidin, which captures CdS-biotin-labeled poly-T-oligonucleotides. Biotin-labeled rRNA-specific capture probes are captured by the quantum dots that are captured by magnetic beads coated with a second 16S rRNA gene capture probe specific for uropathogenic rRNA – when the magnetic bead and the quantum bead bind the same rRNA they are analyzed for Cd (Fig. 1). Metabolite profiling by liquid chromatography mass spectrometry (LC-MS/MS) (Lutz *et al.*, 2008) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Kohling *et al.*, 2012) have also been reported as alternative approaches. The discovery of uncultivable causal organisms in bacteriuria (Wolfe *et al.*, 2012) emphasizes the need for new molecular approaches to aid not only diagnostics for the identification and management of infection but also our understanding of the etiology of bacteriuria.

## Conclusions and future directions

Bacteriuria is among the most globally prevalent infections and a massive financial burden on healthcare costs estimated at US$2.5–3.5 billion in medical expenses and societal costs annually in the USA alone (Nielubowicz & Mobley, 2010; Hannan *et al.*, 2012). Novel molecular tools will drive new discoveries in defining how bacteriuria progresses in different patients and how contemporary culture-based diagnostics can be improved with next-generation detection methods. Cost analyses will be important for new molecular methods to compare with current culture methods. Another crucial aspect (not addressed in this review) is the emerging need to quickly test not only for bacteriuria, but for antibiotic susceptibility. Questions addressed from a clinical viewpoint are better at defining the differences in 'significant' bacteriuria for different organisms in specific patient groups and the implications for diagnostic approaches. Microbiologically, we need to better define the lifestyle adaptations that microorganisms use to aid bacteriuric potential. We also need to understand how ABU due to different bacteria interfaces with the host immune system and how this occurs in different patient populations. Another unanswered question is how ABU due to organisms other than *E. coli* impacts the incidence of sUTI. Finally, we need to investigate whether (and how) noncultivable bacteria may modify the ability of cultivable causal organisms to cause infection and whether ABU can be more effectively applied as a prophylactic probiotic approach to combat sUTI. Future studies will address these key questions.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** ABU prevalence, risk factors and comorbidities in distinct patient populations shown alongside specific prevalence rates of causal microorganisms.