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Microbial Biocatalysis within Us: The Underexplored Xenobiotic Biotransformation Potential of the Urinary Tract Microbiota

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Abstract: Enzymatic biotransformation of xenobiotics by the human microbiota mediates diet-drug-microbe-host interactions and affects human health. Most research on xenobiotics has focused on the gut microbiota while neglecting other body sites, yet over two-thirds of pharmaceuticals are primarily excreted in urine. While microbial biomass and residence time in the gut is higher, xenobiotic biotransformations in the bladder have the potential to affect xenobiotic-microbe interactions including antibiotic resistance and urinary tract infections. However, we have limited knowledge of biotransformations catalyzed by the urinary microbiota. In this perspective, we investigated differences in physicochemical conditions and microbial community compositions between gastrointestinal and urinary tracts. We used a comparative enzyme class mining approach to profile the distribution of xenobiotic-transforming enzyme homologs in genomes of urinary bacteria. Our analysis revealed a discontinuous distribution of enzyme classes even among closely related organisms. We detected diverse amidase homologs involved in pharmaceutical and dietary additive biotransformation pathways, pinpointing microbial candidates to validate for their involvement in xenobiotic transformations in urine. Overall, we highlight the biocatalytic potential of urinary tract bacteria as a lens to study how the human microbiota may respond and adapt to xenobiotic inputs.

Keywords: Artificial sweeteners · Biocatalysis · Genome mining · Gut microbiota · Pharmaceuticals · Urinary tract · Xenobiotics

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A glossary of biological terms is given in the Supplementary Information.

1. Introduction

Xenobiotics are substances either completely foreign to an organism's metabolism or ingested by an organism at unnaturally high concentrations. Xenobiotics include substances such as dietary components, medications, and environmental pollutants (Fig. 1A). When absorbed into the bloodstream, xenobiotics can be biotransformed by liver enzymes, which make xenobiotics more easily excreted through urine. Although xenobiotic biotransformations are mainly studied in the context of the gut microbiota, the urinary system also harbors microbial communities.[1,2] Some xenobiotics like antibiotics alter microbial community composition directly. Microbes are also able to alter xenobiotics through their biocatalytic machinery: enzymes. Bidirectional xenobiotic-microbe interactions can affect human health,[3] for example by increasing antibiotic resistance[4,5] and susceptibility to urinary

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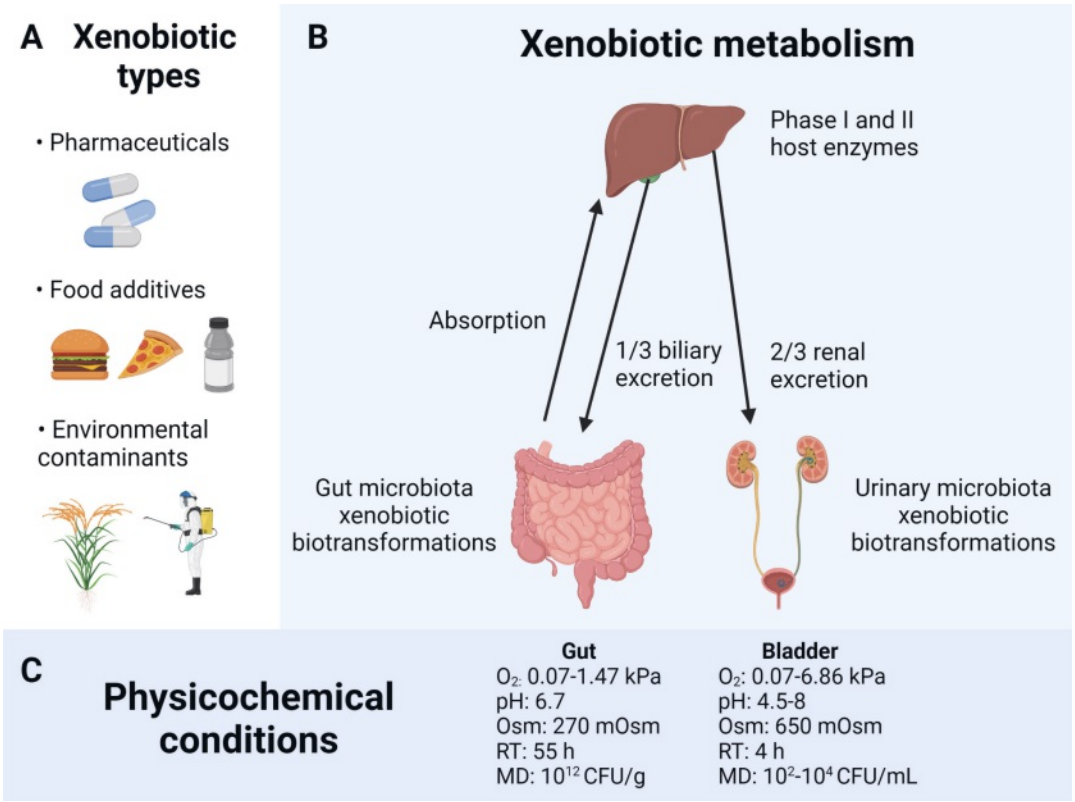

Fig. 1. A) Selection of representative xenobiotics types. B) Xenobiotics are absorbed in the intestine and metabolized in the liver before excretion through the kidneys and gallbladder. Xenobiotics then encounter either the urinary or gut microbiota. Excretion percentages refer to a meta-analysis of 212 pharmaceuticals.[9] C) Physiochemical conditions of the intestine and bladder. Average O_2 partial pressure, pH, Osmolarity (Osm), residence time (RT) and microbial density (MD) based on Neugent *et al.* (2020) and references in section 1.3.[10] Image created with BioRender.com. For more background, a brief review section of physiological consequences of xenobiotic biotransformation by the gut microbiota is available in the Supplementary Information.

tract infections.[6] In this perspective, we investigate the xenobiotic biocatalytic potential of the urinary microbiota.

Upon entering the systemic circulation of an organism, xenobiotics reach the liver which increases hydrophilicity of the xenobiotics by unmasking or introducing polar functional groups, and by forming conjugates with more polar compounds. These conjugated xenobiotics are excreted mainly in urine *via* the kidneys or the bile of the gallbladder (Fig. 1B).[7,8] An analysis of human excretion of the active ingredients in 212 pharmaceuticals found on average, two-thirds of each active ingredient was excreted through urine and one-third through feces. Analgesics, such as paracetamol and acetylsalicylic acid, and antiepileptic drugs such as gabapentin are excreted almost exclusively in urine.[9]

1.1 *The Urinary Microbiota is Exposed to the Majority of Excreted Xenobiotics*

Xenobiotics excreted through the feces and urine come in contact with the gut and urinary bacteria. With the advent of microbiome research, it became clear that host-associated bacteria can also biotransform xenobiotics.[11,12] In general, gut bacteria tend to transform chemicals by reduction or hydrolysis, unlike the human host xenobiotic metabolism which favors oxidation and the conjugation of hydrophilic groups.[3] While xenobiotic microbial transformations have been characterized in detail for the gut microbiota, research on the urinary microbiota xenobiotic metabolism is still limited.

Little is known about biotransformations in the urinary tract because it was long considered to be sterile.[1] This false assumption was disproven with the development of enhanced quantitative urine culture (EQUC), which allowed isolation of a larger variety of species from urine, and by DNA sequencing techniques.[13–15] Urinary bacteria are thought to originate from other body sites, in particular the gut and the vagina. Of the overall 672 species that could be cultured across multiple urine samples,[16] 64% were previously found in the gut, 32% in the vagina, and 40% in the oral-respiratory tract. Interestingly, 140 species have been found exclusively in urine.[16] Typically, the urinary microbiota of an individual is characterized by the dominance of a single genus, which is termed the urotype. The most frequent urotype in women is dominated by *Lactobacillus*.[2,15] This is likely due to the proximity to the vagina which harbors high densities of lactobacilli.[17] Urotypes in men are harder to define since the male urinary microbiota is understudied and sample sizes were small, however *Corynebacterium*, *Streptococcus* and *Staphylococcus* are common genera in men.[2,15,18] To compare the composition of the urinary microbiota with that of the gut and vaginal microbiota, we used a dataset published by Biehl *et al.* based on amplicon sequencing of the V3-V4 region of 16S rRNA from all three body sites (Fig. 2).[19] Vaginal samples were collected using a vaginal swab, while urinary samples were collected by catheter. A detailed description of the sampling procedure can be found in Biehl *et al.*,[19] and details of our analysis can be found in the Methods in the Supplementary Information. In agreement with other studies,[16] Biehl *et al.* observed a high compositional similarity between the urine and vaginal microbiota where *Lactobacillus* is the dominant genus. *Gardnerella* also has a high relative abundance at both sites. The gut microbiota is more diverse overall, with *Bacteroides* and *Faecalibacterium* standing out as the most abundant genera.[19]

These taxonomic differences highlight the need to characterize the urinary microbiota relative to the gut. Despite the similarity in taxonomic composition between the vagina and urinary tracts, ingested xenobiotics do not pass through the vagina in notable quantities. Therefore, in this perspective we focus on comparisons between the gut and urinary tract.

When xenobiotics come into contact with the human microbiota, their metabolism can influence microbial community structure in several ways. Bacteria that are able to use xenobiotics as a carbon or energy source gain a growth advantage. For example, beneficial gut bacteria *Lactobacillus* and *Bifidobacterium* are capable of utilizing galacto-oligosaccharide supplements through β-galactosidase enzymes, thereby gaining a growth advantage over bacteria that do not have or express these enzymes.[20–22] Some xenobiotics can also have an inhibitory effect on the growth of certain bacteria. Antibiotics are the most prominent example,

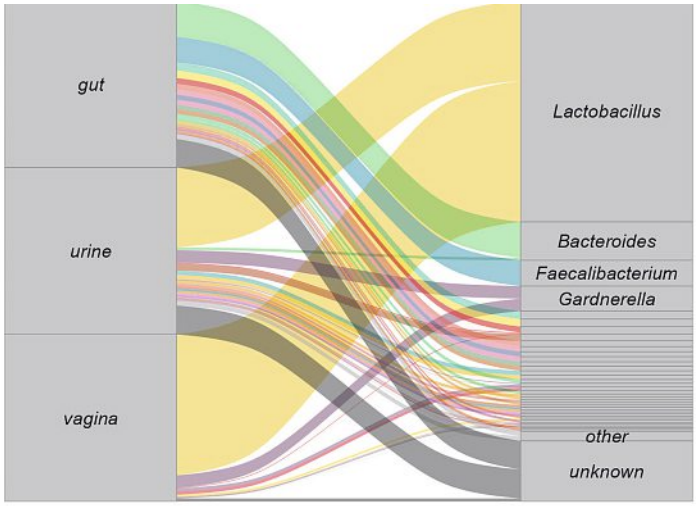

Fig. 2. Comparison of the microbiota composition in the gut, urine, and vagina with colored lines corresponding to microbial taxa classified at the genus level, with the top four most abundant taxa across sites labeled on the right. Line widths correspond to the mean relative abundance of each genus in each body site. Data are based on 16S rRNA abundances from samples collected from 15 women, in data previously published by Biehl *et al.*[19] Taxa with a relative abundance of <0.01% were categorized as 'other', while all amplicon sequence variants which could not be classified at the genus level were categorized as 'unknown'.

but also other xenobiotics such as artificial sweeteners and antidepressants inhibit bacterial growth and promote antibiotic tolerance and persistence, respectively.[5,23] While these effects have been studied in gut bacteria, similar principles may apply but await characterization in the urinary tract.

1.2 *Physicochemical Conditions Differ between the Gut and Bladder*

Physicochemical factors differ considerably between the gut and bladder (Fig. 1C). As a result, we expect diverse microbial xenobiotic metabolism to occur at the two body sites. Gut and bladder microbiota vary in their composition and their exposure to xenobiotic compounds with different hydrophilicities and molecular weights.[24,25] Moreover, bacteria migrating from the gut, vagina or the pelvic area to the bladder need to adapt to the new environmental conditions. Here we review carbon availability, residence time, microbial density, pH, osmolarity and oxygen content in the gut and urinary tract, and highlight implications for xenobiotic biotransformations in urine.

1.2.1 *The Nutrient Composition of Urine Affects Microbial Metabolic Strategies*

The urinary tract is nutrient-limited relative to the gut, reflected by the higher energy content of stool compared to urine.[26] The colon receives large amounts of undigested carbohydrates, also known as fibers. Fibers, alongside mucus glycoproteins and glycans are used as carbon sources by gut bacteria.[27] In contrast, carbon to support bacterial growth in the bladder is typically obtained through degradation of peptides found in urine and by scavenging mucosal sugars.[28,29] Therefore, microbes entering the urinary tract require metabolic adaptations to survive.[29,30] For example, when uropathogenic *Escherichia coli* (UPEC) migrates from the gut or vagina to the bladder, it adapts its central carbon and amino acid metabolism to utilize amino acids, nucleic acids and other compounds found in urine.[28,29] These metabolic adaptations were so far mainly characterized in UPEC as the most clinically-relevant uropathogen causing over 75% of urinary tract infections (UTIs).[31] Other bacteria entering the urinary system are also likely to undergo metabolic adaptations. The urinary system also receives additional carbon in the form of xenobiotics, but the extent that these 'foreign' carbon sources are used to support growth is poorly understood.

1.2.2 *Lower pH and Higher Osmolarity likely Alter Enzyme Activity in Urine*

Osmolarity and pH are also important factors which vary between the gut, vagina and urinary tract. The vagina is the most acidic of the three environments (pH 3.5–5) given the lactic acid production by *Lactobacillus* which dominates the vaginal microbiota.[17,32,33] The colonic pH is around 6.7,[34] while the pH of urine of healthy individuals is on average ~6 and ranges from 4.5 to 8, depending partly on diet and hydration levels.[35,36] The osmolarity of urine is ~650 milliosmoles (mOsm),[37,38] while that of stool and vaginal fluids is around 290 and 370 mOsm, respectively.[39,40] Osmolarity and pH can affect how proteins are folded and hydrated,[41] which in turn affects their stability and activity with substrates including xenobiotics.

1.2.3 *Microbial Densities and Xenobiotic Residence Times May Affect Biotransformation Rates*

Another key difference between the urinary tract and gut microbiota is microbial cell density. While the gut microbiota can harbor 10^{12} colony forming units (CFU) per gram feces, the urinary microbiota typically has around 10^2–10^4 CFU per milliliter (mL) urine.[2] The host immune system also influences the bacterial density and community structure depending on the body site. In the gut and vagina, high cell densities are tolerated by the immune system, while microbial immune tolerance for the urinary microbiota has not been investigated.[17,42,43]

Not only are xenobiotics in the gut exposed to higher bacterial densities, but they are also exposed to them for longer. The transit time for renally excreted molecules including xenobiotics in the bladder is, on average, 4 hours,[44] while compounds spend approximately 55 hours in the intestine.[45] Xenobiotic residence time is therefore shorter in the bladder compared to the intestine. However, biotransformations are likely facilitated by the higher rate of diffusion of xenobiotics in urine over stool. Moreover, the constant filling and emptying of the bladder, together with the shear force exerted by the flow of urine during urination also likely favors the formation of microbial biofilms.[46] Biofilms are composed of extracellular polymeric substances that also allow microbes to persist in systems with liquid flow.[47] While the presence of biofilms in the bladder of healthy asymptomatic individuals has not been investigated, several species, in particular *E. coli* strains with genetic makeup (including adhesion factors) similar to uropathogenic *E.coli*, notorious for its ability to form biofilms, have been found in the urinary microbiota of healthy asymptomatic women.[48] Moreover, biofilms create chemical gradients that decrease the efficacy of antibiotic treatment and drive metabolic specialization,[49,50] such as xenobiotic catabolism.

1.2.4 *Higher Oxygen Concentration in the Bladder likely Allows for Oxidative Biotransformations*

Oxygen concentration also varies between the urinary tract and the gut. The gut microbiota encodes a high number of oxygen-sensitive enzymes involved in xenobiotic biotransformations including glycyl radical and radical *S*-adenosylmethionine enzymes.[12,51] In urine, a partial pressure of oxygen up to 6.86 kPa has been detected, about 5–100 times higher than the range measured in the colon.[52–55] With respect to xenobiotic biotransformations, many unfavorable reactions under anaerobic conditions would be favorable in the urinary tract in the presence of oxygen. However, the activity of redox enzymatic reactions under conditions in the urinary tract has not been analyzed experimentally.

Since experimental data on xenobiotic biocatalysis by the urinary microbiota is limited, we found insufficient existing literature on the topic to review. Therefore, in the next section, we performed new bioinformatic analysis of the xenobiotic biotransformation potential of urinary bacteria isolates and highlighted directions where future research is needed.

2. Results and Discussion

2.1 *Predicted Biotransformations of Xenobiotics Commonly Excreted in Urine*

Extrapolation of urinary microbial xenobiotic metabolism based on research in the gut microbiota is challenging given key differences in physicochemical conditions and community composition. To investigate the latter point, we used a genome mining approach to evaluate the distribution of enzyme classes in bacteria prevalent in the urinary tract. We selected a study set of ten xenobiotics (Fig. 2), based on the following criteria: 1) compounds frequently detected in urine, 2) with >80% relative urinary excretion (Supplementary Information, Table 1), and 3) spanning different chemical classes of pharmaceuticals and dietary components (Fig. 3A).

We used both gut microbiota-specific (DrugBug)[56] and more generalized microbial biotransformation (enviPath) prediction tools[57] to link these ten xenobiotics with their likely biotransformations based on chemical functional groups (Supplementary Information, Table 2). Overall, most Enzyme Commission (EC) numbers for predicted biotransformations using DrugBug were also predicted by enviPath (Fig. 3B). Details of our bioinformatic analyses are described in the Supplementary Information, Methods.

2.2 *Bacillus and Gordonia have the Highest Biotransformation Potential*

We analyzed the genomes of urinary bacteria isolated from catheter urine of women using the EQUC protocol[59] (Supplementary Information, Table 3) for enzyme classes (Supplementary Information, Table 4) predicted to be involved in biotransformations of our study set xenobiotics (Fig. 3A). A detailed description of the analysis can be found in the Supplementary Information, Methods. Our analysis revealed a discontinuous distribution of enzyme classes even within closely-related species belonging to the same genus *e.g.*, *Streptococcus, Lactobacillus,* and *Corynebacterium* (Fig. 4). *Bacillus* sp. UMB0893 and *Bacillus* sp. UMB0728 had the broadest range in its distribution of predicted EC numbers, spanning ten and eight different EC numbers, respectively. The urinary actinomycete, *Gordonia terrae* UMB0777, encodes the highest number of oxidoreductases. These results particularly highlight the broad biotransformation potential of urinary *Gordonia* and *Bacilli*, supported by the established roles for these clades in industrial biocatalysis and xenobiotic metabolism.[60,61]

2.3 *Oxidoreductases and Hydrolases are Conserved in Urinary Bacterial Genomes*

Biotransformation rules triggered in our analysis corresponded to EC classes within the oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC 3). Among the oxidoreductases, homologs of NADP-dependent oxidoreductases/dihydropyrimidine dehydrogenases (EC 1.3) were the most widely distributed in urinary bacterial genomes. These characterized reference proteins are subunits of a heterotetrameric enzyme that reduces uracil and also promiscuously catalyze the reduction of pharmaceuticals such as 5-fluorouracil[62] discussed further in Section 3.4. Transferases (EC 2) were only sparsely distributed. Among the hydrolases (EC 3), amidases known to cleave paracetamol[63] and acesulfame transformation products[64] were the most abundant hydrolases detected in urinary bacterial genomes (Fig. 4). Based on this broad bioinformatic analysis, we selected four xenobiotics and their predicted biotransformations as case studies (Section 3).

3. Case Studies

3.1 *Sulfamethoxazole*

One of the most commonly prescribed treatments for UTIs is a combination of trimethoprim (TMP) and sulfamethoxazole (SMX), with SMX comprising 80% of the drug composition.[65] Approximately two-thirds of SMX is excreted in a conjugated form *e.g.*, N^4-acetyl-SMX (Fig. 5).[66] Pterin conjugation is an additional major SMX microbial biotransformation route, at least in wastewater.[67] SMX stress was also recently shown to induce human gut bacteria to produce pterin-containing metabolites,[68]

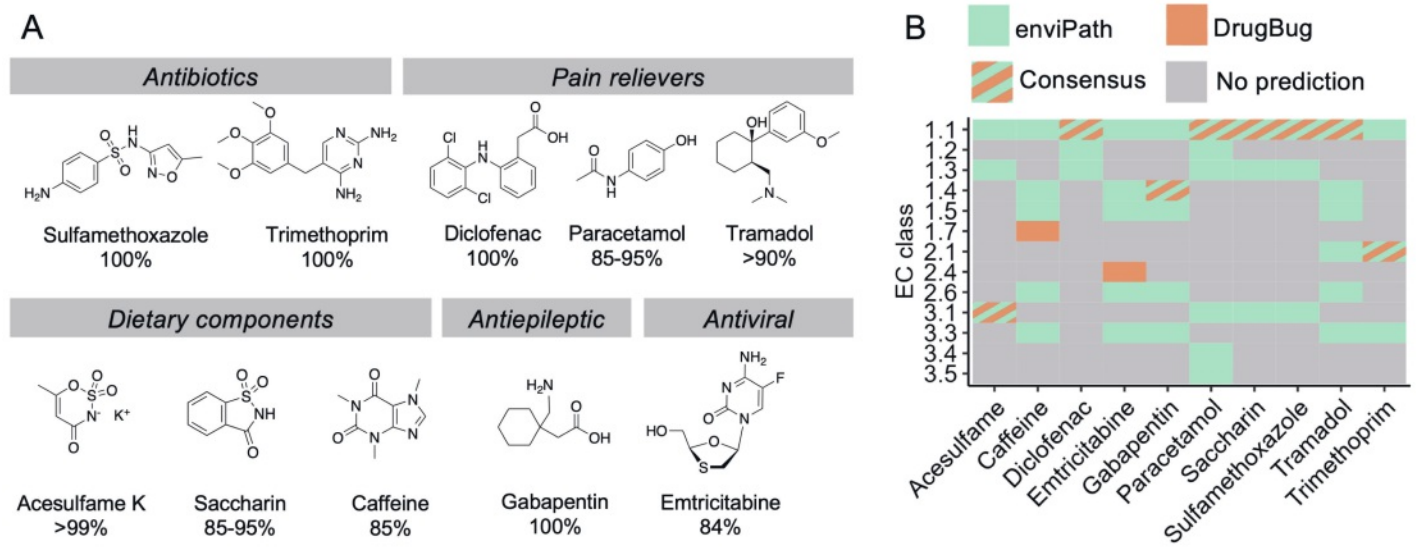

Fig. 3. A) Xenobiotics included in this study. Percentages of excretion in urine relative to stool are indicated under the compound names with references in the Supplementary Information, Table 1. B) For the ten xenobiotics in our study set, second level Enzyme Commission (EC) numbers are displayed which correspond to biotransformation rules triggered by the microbial biotransformation prediction tool enviPath.[57,58] For comparison, we queried the same compound set against the gut microbiota-specific tool, DrugBug.[56] DrugBug predictions (limited to one EC number prediction per compound) were in consensus with enviPath for eight out of ten xenobiotics.

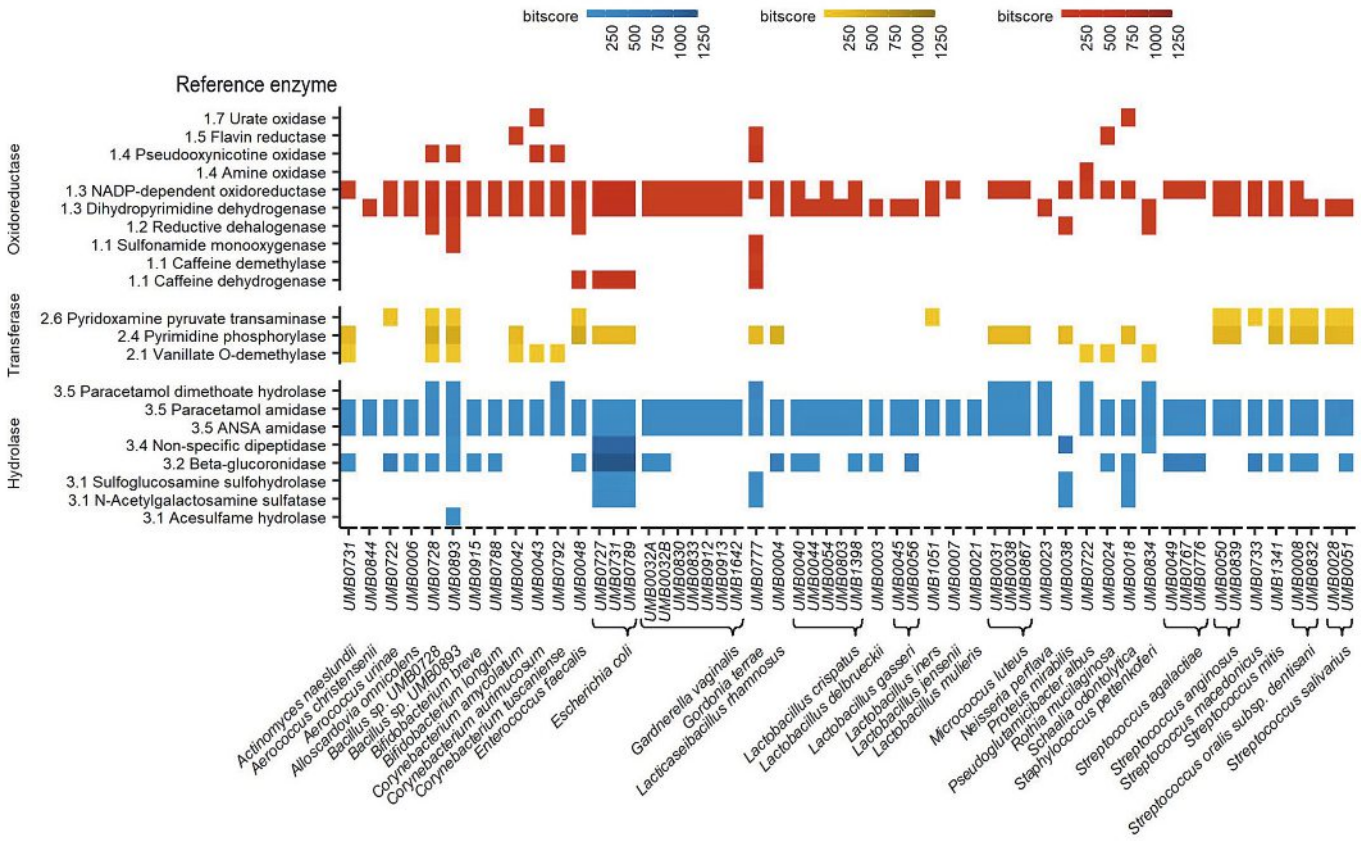

Fig. 4. Homologs of reference enzyme sequences (Supplementary Information, Table 4) corresponding to three different top level EC numbers: oxidoreductases (EC 1, red), transferases (EC 2, yellow), and hydrolases (EC 3, blue) encoded in microbial genomes (Supplementary Information, Table 3) isolated from the urinary tract of women. The sequence alignment quality (bitscore) of the top hit within each genome is indicated by color intensity. Hits in urinary bacterial genomes share sequence identity with reference enzymes but their functions or substrates cannot be verified without experimental evidence.

suggesting deeper investigation is needed into SMX conjugation pathways in the urinary tract.

SMX biotransformation *via ipso*-hydroxylation (Fig. 5) and subsequent transformation steps enable bacteria to grow on SMX as a carbon source.[6,69] The *sadABC* gene cluster coding for SMX biodegradation was first characterized in *Microbacterium* and *Arthrobacter,* both previously isolated from urine and able to degrade SMX in artificial urine medium.[6,69] Our genomic analysis additionally detected a cluster of genes encoding essential SMX biotransformation enzymes SadA (EC 1.1, sulfonamide monooxygenase) and SadC (EC 1.5, flavin reductase) in the urinary isolate *Gordonia terrae* UMB0777 (Fig. 4). The SadA monooxygenase, with a partner flavin reductase SadC cleaves SMX into 3-amino-5-methylisoxazole and 4-aminophenol (Fig. 5). Notably, the *sadA* and *sadC* genes are clustered within the *Gordonia terrae* UMB0777 genome, as in *Microbacterium* sp. CJ77, where the cluster was originally characterized.[6]

Subinhibitory antibiotic concentrations as a result of microbial SMX biodegradation could facilitate the emergence of antibiotic resistance.[70,71] Moreover, antibiotics used to treat UTIs affect the composition of the urinary microbiota by killing sensitive microbes. More research is needed to understand how SMX biotransformation may alter the structure of urinary microbiota, promote antibiotic resistance, or alter the efficacy of UTI treatments.

3.2 *Paracetamol*

The widespread pain reliever paracetamol (acetaminophen) and its conjugation products are the most abundant xenobiotics detected in urine[72] with a median concentration around 60 µg/L and detection in some patients up to the g/L range.[73] Paracetamol is almost exclusively excreted in urine, primarily in its glucuronidated or sulfated forms.[74] Gut bacteria produce *para*-cresol that competes with paracetamol for modification by a host sulfotransferase SULT1A1.[75,76] Blocked SULT1A1 active sites can result in paracetamol being instead converted by host cytochrome p450 enzymes into a toxic byproduct, *N*-acetyl-*p*-benzoquinone imine.[75,76] The interplay between bacteria and host thus directly affects paracetamol efficacy and toxicity.

In addition to bacterial cleavage of conjugated drug forms by sulfatases (EC 3.1) and β-glucuronidases (EC 3.2), microbial paracetamol amidases can also directly cleave paracetamol into 4-aminophenol and acetate for use as a carbon source.[63] Amidases (EC 3.5) capable of cleaving paracetamol are remarkably diverse with as low as 31% amino acid identity between related enzymes and even lower identity between different paracetamol amidase enzyme families.[63] In our analysis, amidase signature family enzymes were the most widely-distributed enzyme class, with homologs encoded in ~98% of the urinary bacterial genomes we analyzed. Further investigation is required to understand whether enzymes acting on paracetamol and its transformation products may provide access to paracetamol as an energy source for urinary bacteria in a nutrient-limited bladder environment.

3.3 *Acesulfame K*

Acesulfame K (ACE), an artificial sweetener, has been detected with an average concentration of 4070 µg/L in urine.[77] Once ingested, ACE is rapidly absorbed in the small intestine, absorbed in the systemic circulation and quickly excreted through urine without undergoing liver enzyme conjugations.[78] ACE is >99% excreted in the urine and <1% in the feces (Supplementary Information, Table 1). Nonetheless, ACE has mainly been investigated with respect to the gut microbiota.[79,80] Studies by Yu *et*

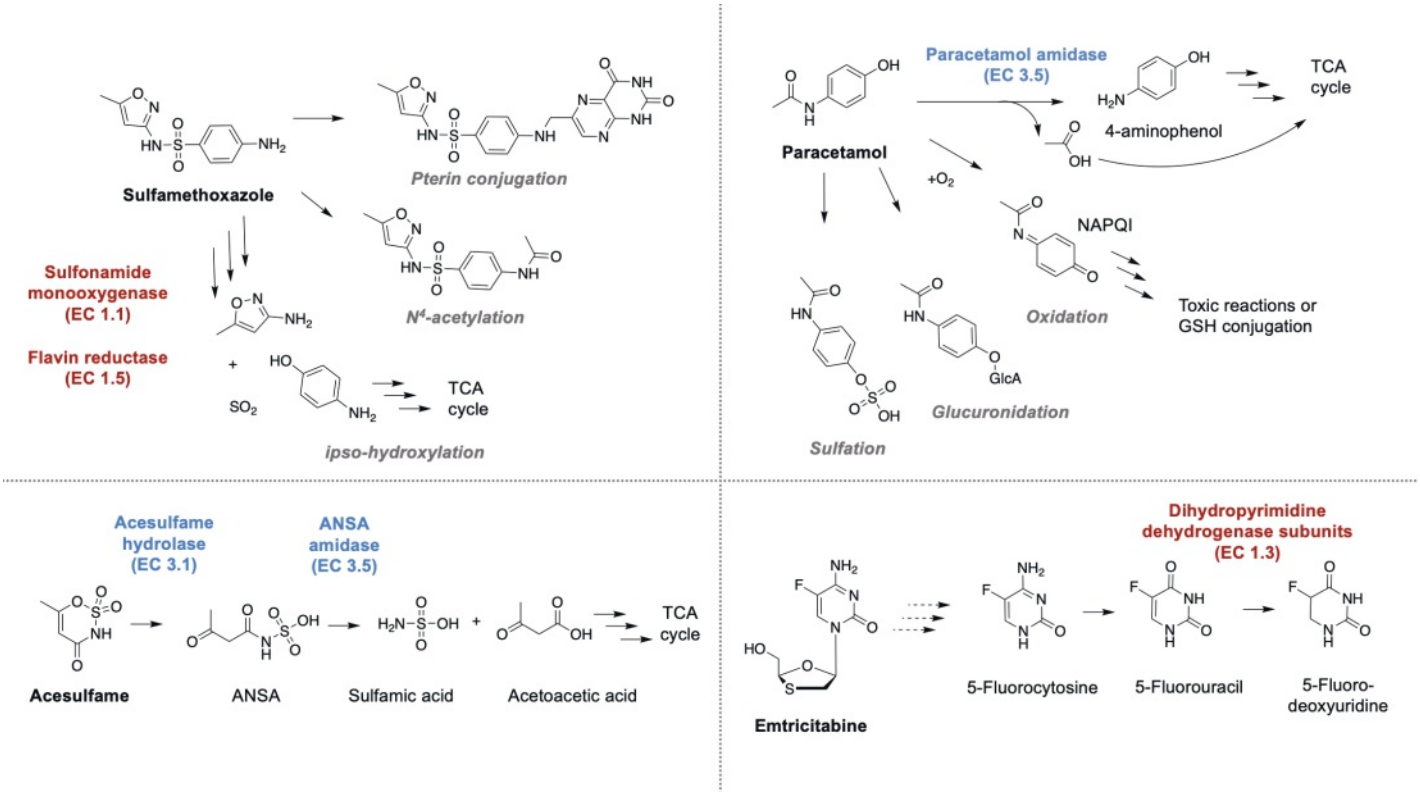

Fig. 5. Case studies of microbial xenobiotic transformations and predicted EC numbers. Text coloring corresponds to the EC numbers oxidoreductases (EC 1, red) and hydrolases (EC 3, blue). GlcA = glucuronic acid, NAPQI = N-acetyl-p-benzoquinone imine, ANSA = acetoacetamide *N*-sulfonate. Reactions represent only a fraction of possible biotransformations, highlighting the major pathways discussed in this review., Of note, conjugates can be reactivated back to their parent compound state *e.g.*, by microbial β-glucuronidases and sulfatases.

al. in mouse fecal samples and *in vitro* model systems suggested ACE in the mg/L range may promote the spread and direct uptake of antibiotic resistance genes.[4,79] The potential for ACE and other artificial sweeteners to promote antibiotic resistance has not been evaluated in the urinary tract where ACE concentrations are significantly higher than in the gut.

In the human body, ACE is recalcitrant to metabolism.[81] Evidence for microbial ACE biotransformation in wastewater treatment plants was first reported within the last decade.[82–84] Microbial enzymes responsible for the ACE ring-opening reaction (Fig. 4, EC 3.1 ACE hydrolase) were recently identified in Alphaproteobacteria including *Bosea* and *Chelatococcus*.[64,85] Cleavage of the stable ACE ring structure is catalyzed by an ACE hydrolase (EC 3.1) in the metallo-β lactamase (MBL) family of enzymes.[64] Only a single ACE hydrolase homolog with low (26.3%) amino acid identity was detected in our set of urinary bacterial genomes in *Bacillus* sp. *UMB0893*. In such a low sequence identity range, the substrate specificity of this enzyme is not known. Downstream amidases acting on the major ACE transformation product, acetoacetamide-*N*-sulfonate (ANSA)[64,86] have a much broader distribution in urinary tract bacteria (Fig. 4., EC 3.5, ANSA amidases). Given the mg/L concentrations of ACE detected in urine, further research is necessary to determine if and how the potential for ACE metabolism could evolve in members of the urinary microbiota.

3.4 *Emtricitabine*

Emtricitabine is a virostatic compound detected in the µg/L range in wastewater and urine.[87] Emtricitabine is a fluorinated cytosine analog which acts by inhibiting RNA reverse transcriptase; it is widely used in combination therapy for HIV pre-exposure prophylactic treatment. Emtricitabine's persistence and high urinary excretion qualifies its use as a real-time marker to assess patient adherence to treatment.[88,89]

Emtricitabine's biotic and abiotic transformation products[90] include fluoropyrimidine metabolites similar to the widely-used anticancer drug 5-fluorouracil and its analogs (Fig. 5). Recently, researchers identified a conserved iron-sulfur flavoenzyme in gut bacteria which reduces 5-fluorouracil to 5-fluorodeoxyuridine, resulting in drug inactivation and reduced treatment efficacy.[62] Interestingly, PreA and PreT (EC 1.3, annotated as NADP-dependent oxidoreductases and dihydropyrmidine dehydrogenases, respectively) forming a heterotetramer responsible for fluoropyrimidine inactivation, are also conserved in >75% of urinary bacterial genomes in our study (Fig. 4). Although further studies on the substrate range are necessary, our analysis supports the wide distribution of fluoropyrimidine catabolic enzymes[62] in the urinary tract. Specifically, assessment of whether microbial biotransformations may influence the measured amounts of emtricitabine and other fluoropyrimidines in urine is relevant for the assessment of point-of-care diagnostics.

4. Conclusions and Outlook

Microbial xenobiotic biocatalysis has important physiological and pharmacological effects on the human body primarily based on studies in the gut, while the urinary tract has been largely overlooked. Microbes in the gut and urinary tract experience different environmental conditions which alter community structure and functional profiles across the two body sites. Here we explored the biocatalytic potential of urinary bacteria through xenobiotic biotransformation predictions and comparative genomics. Through case studies, we examined urinary microbial biotransformations in the context of antibiotic resistance, UTIs, and point-of-care diagnostics, and highlighted the need for further observational and experimental validation. Future research integrating various 'omics approaches will deepen our understanding of how xenobiotic pressures shape the urinary microbiota. The considerable influence of xenobiotics on our microbial inhabitants should be

taken into account in order to develop safe and effective therapeutics, food additives and diagnostics.

Supplementary Information

Supplementary Information including a glossary for definitions of biological terms, a description of methods and additional information is available at https://doi.org/10.2533/chimia.2023.424.

Data and Code Availability

Scripts and associated data for biotransformation prediction and genomic analysis are available at github.com/MSM-group/urinary-biotransformations.

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Author contributions

Conceptualization – T.D.M., S.L.R.; Data curation – T.D.M., M.R.S., S.L.R.; Formal analysis – T.D.M., M.R.S., S.L.R.; Software – M.R.S.; Funding acquisition – S.L.R;, Visualization – T.D.M., M.R.S., S.L.R.; Writing – T.D.M., M.R.S., S.L.R.

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