MiniReview

Escherichia coli mediated urinary tract infections: Are there distinct uropathogenic *E. coli* (UPEC) pathotypes?

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Abstract

A variety of virulence genes are associated with *Escherichia coli* mediated urinary tract infections. Particular sets of virulence factors shared by bacterial strains directing them through a particular pathogenesis process are called a "pathotype." Comparison of co-occurrence of potential urinary tract infection (UTI) virulence genes among different *E. coli* isolates from fecal and UTI collections provides evidence for multiple pathotypes of uropathogenic *E. coli*, but current understanding of critical genetic differences defining the pathotypes is limited. Discovery of additional *E. coli* genes involved in uropathogenesis and determination of their distribution and co-occurrences will further define UPEC pathotypes and allow for a more detailed analysis of how these pathotypes might differ in how they cause disease.

1. Introduction

Escherichia coli are a very diverse species of bacteria found naturally in the intestinal tract of all humans and many other animal species. A subset of *E. coli* are capable of causing enteric/diarrhoeal disease, and a different subset cause extra-intestinal disease, including urinary tract infection (UTI). The determination of six different *E. coli* "pathotypes" that cause enteric/diarrhoeal, enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC), greatly enhanced our understanding of pathogenic *E. coli*. Each pathotype causes disease using different combinations of virulence factors, with different molecular pathways, and in some but not all cases resulting in disease symptoms that can be distinguished from each other (for review see [1]). This improved understanding of *E. coli* causing enteric/diarrhoeal disease has been very useful relative to therapeutic and vaccine development.

By contrast, pathotypes have yet to be distinguished for UTI, although by analogy, a designation 'uropathogenic *E. coli*' (UPEC) is in common usage. UTIs are one of the most frequently acquired bacterial infections [2] and *E. coli* accounts for as many as 90% of all UTIs seen among ambulatory populations [3]. Overall, approximately half of all women have had a UTI by their late 20s [2]. About 20–30% of women with first UTI will have two or more infections [4]; and, for 5%, chronic recurring infections which greatly disrupt a woman's life

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[5]. In the United States, the annual total direct and indirect costs of UTI in 1995 were estimated to be \$1.6 billion as the result of urinary infections suffered by an estimated 11.3 million women [2].

A UTI is defined as a significant number of pathogenic organisms in the urinary system. If symptoms, such as painful or frequent urination or blood in the urine, are present, as few as 100 uropathogenic bacteria per milliliter urine may be considered significant [6]. Bacteria can be detected at high concentrations in the urinary tract in individuals during routine urine examination. However, many of these individuals experience no symptoms. This condition is termed asymptomatic bacteriuria (ABU). Cases of symptomatic bacteriuria are classified either as cystitis (CY) when infection is limited to bladder or pyelonephritis (PY) when the kidney is infected [7]. While cystitis in the otherwise healthy individual generally resolves without sequelae, pyelonephritis can cause serious morbidity and can be fatal. Patients with abnormal or obstructed urinary tracts or with compromised immune systems are at high risk of UTI. These infections are often referred as complicated UTIs. There is an increased risk in this group that a simple urinary tract infection may progress to systemic infection.

Unlike enteric/diarrhoeal diseases that often occur in clear epidemiologic clusters, UTI epidemics are, as yet, difficult to identify. The high background rate of UTI [2], the impression that UTI among ambulatory patients is caused solely by auto-infection, and the great diversity of *E. coli* obscures potential epidemics. To the best of our knowledge, only one UTI outbreak has been reported among ambulatory patients. In Greater Copenhagen, Olesen et al. identified a cluster of multi-resistant, lactose-negative *Escherichia coli* O78:H10 causing urinary symptoms among 14 community members. Only the unusual lactose-negative phenotype made the cluster visible [8]. This lack of epidemiologic clusters has made it much more difficult to determine if there are separate pathotypes of UPEC.

Several recent reviews give detailed overviews of UPEC pathogenesis factors [1,9–12]. Here, we present a brief summary of what is currently known about UPEC virulence genes, and then examine the evidence for the existence of distinct UPEC pathotypes with different sets of virulence genes that help determine the specific *E. coli*–host interactions that lead to urinary tract infections.

2. Summary of currently studied UPEC virulence genes

Table 1 presents a set of *E. coli* genes that have been potentially implicated as important in allowing some UPEC isolates to establish urinary tract infections. The first one we will discuss is the *fim* gene cluster that encodes the proteins responsible for type 1 pilus production. Unlike the other genes in Table 1, the *fim* genes are not present in a statistically significantly higher set of UPEC vs. rectal isolates as almost all *E. coli* have the *fim* genes. However, as is true with many of the pilus gene clusters in *E. coli*, type 1 pilus expression is regulated in part by a phase-variation system. Gunther IV et al. [19] compared the fate of mutants phase-locked in either the on or off configuration in a mouse model of ascending urinary tract infection and found that the locked-off mutants were recovered from the urine, bladder and kidneys 24 h post-inoculation in significantly

Table 1
Putative uropathogenic *E. coli* (UPEC) virulence factors and proposed function or homology

Genes (virulence factor)	Function or homology	Reference[a]
cnf1 (cytotoxic necrotizing factor 1)	Causes multinucleation and rounding of human cells	[13–15]
cvaC (colicin V)	Colicin	[16]
drb (Dr family of adhesins)	Adherence factors that bind decay acceleration factor (DAF)	[13,17,18]
fim (type 1 pili)	Adherence to uroepithelial cells and FimH mediated invasion	[19–21]
hly (α-hemolysin)	Lyses red blood cells	[13,22,23]
hra (heat-resistant agglutinin)	Adherence factor	[24]
iha (IrgA homolog adhesin)	Adherence factor	[25–27]
iroN$_{E.\ coli}$ (IroN$_{E.\ coli}$)	Siderophore receptor	[25,27,28]
iucD (aerobactin)	Siderophore that binds iron	[13,29]
kpsMT (group II capsule)	Capsular polysaccharide production	[13,25]
ompT (OmpT)	Outer membrane protease	[13,25]
papG$_{AD/IA2}$ (class II P-pili)	Adherence to uroepithelial cells	[10,13,30]
prsG$_{J96}$ (class III P-pili)	Adherence to uroepithelial cells	[10,13,30]
picU (PicU)	Homolog to Pic (protein involved in intestinal colonization) serine protease autotransporter	[31,32]
sat (Sat)	Serine protease autotransporter toxin	[33,34]
sfa (S-fimbrial family)	Adherence factors	[13,35]
usp (uropathogen specific protein)	Bacteriocin	[25,27,36]
vat (Vat)	Vacuolating autotransporter toxin	[37,38]
PaiII$_{cft073}$	Multiple genes	[39,40]

[a] Due to space limitations, only up to three references purporting UTI virulence association for each gene are listed.

lower numbers than the wild type, while the locked-on mutants were recovered at higher numbers than the wild type, implying an important role for type 1 pili in initial colonization of the bladder. In addition to mediating adherence to bladder epithelium, the FimH adhesin at the tip of type 1 pili mediate invasion of bladder epithelial and mast cells into caveolae [20,21], which has been proposed to protect the bacteria from host defenses and antibiotics. This may explain the observation that roughly one half of all second UTIs are caused by the same E. coli strain that caused the initial UTI [41,42].

One of the most studied groups of UTI virulence factors is the P-fimbrial adhesins that mediate attachment to the P-blood group antigens on uroepithelial cells. The P-fimbrial-tip adhesin is encoded by the papG gene, and four classes of these adhesin genes have been described that have between 45% and 65% homology to each other at the amino acid level [30]. Only two of these four classes, $papG_{AD/IA2}$ (class II) and $prsG_{J96}$ (class III), are clearly associated with uropathogenicity [13,30]. Class II adhesin genes are found predominantly among pyelonephritis isolates, while class III adhesin genes occur somewhat more frequently among cystitis isolates than among pyelonephritis isolates [13]. Colonization studies of human volunteers with isogenic strains with and without either class II or class III P fimbriae have demonstrated that P fimbriae improve E. coli colonization of the bladder and also increase the host immune response to the bacteria [10]. P fimbriae trigger the Toll-like receptor 4 pathway for host cell activation [43].

The Dr family of adhesins share a common receptor, the Dr blood group antigen component of decay-accelerating factor [17]. This widely distributed receptor along the urinary tract may underline the potential importance of Dr adhesin-producing E. coli in ascending colonization of the urinary tract. Dr adhesins occur more frequently among E. coli cystitis isolates than fecal isolates [13]. We also found the presence of Dr at time of first UTI is associated with an increased risk of second UTI [41]. In general there are no significant difference in the prevalence of Dr adhesins in pyelonephritic isolates when compared to fecal isolates [13]; however, Goluszko et al. [18] found that 30–40% of E. coli isolates from pregnant women with pyelonephritis had Dr operons.

S fimbriae of E. coli bind to sialyl galactosides and are implicated in experimental UTI in rats [44]. UTI isolates are at least two times more likely to carry S fimbriae genes than fecal isolates [13,35]. More recently, two additional potential adherence factors, encoded by the genes hra and iha, were associated with UPEC isolates. The hra gene was found in 43–58% of cystitis isolates, and in 66% of pyelonephritis isolates, compared to just 28% of rectal isolates [24]. There are mixed reports on the potential importance of iha. Kanamaru et al. [25] compared 194 cystitis, and 76 pyelonephritis isolates to 50 fecal isolates from healthy adults and found iha pres-

ent in 39% of cystitis, 45% of pyelonephritis, and only 12% of fecal isolates. Johnson et al. [26] looked at the prevalence of iha in a variety of E. coli, finding it present in 13/77 (17%) of fecal isolates from healthy women, 34/156 (22%) of cystitis isolates from women, 97/170 (57%) of pyelonephritis isolates from women, and 25/48 (52%) of pyelonephritis isolates from children, where the later two sets were statistically significantly different from the fecal frequencies. By contrast, Bauer et al. [27] found iha in 64/184 (35%) of fecal isolates from healthy women, 129/355 (36%) of cystitis isolates from women, and 61/153 (40%) of pyelonephritis isolates from children, with no significant differences between the fecal isolates and the UTI ones. Clearly, the major difference between the Kanamaru et al. and Johnson et al. reports and the Bauer et al. one is in the frequency of iha in the fecal isolates from healthy women, something that future studies may resolve. Johnson et al. [26] also have shown that an iha deletion mutant of CFT073 is out competed by CFT073 in the mouse model of ascending UTI.

Toxins are important virulence factors in a variety of E. coli mediated diseases. Production of toxins by colonized E. coli may cause an inflammatory response, a possible pathway for UTI symptoms. Two toxins associated with uropathogenic E. coli are α-hemolysin (HlyA) and cytotoxic necrotizing factor 1 (CNF1). In 1991, O'Hanley et al. reported that α-hemolysin was correlated with septicemia and renal damage in a mouse model of pyelonephritis [22]. In 1993, Ikaheimo et al. [23] reported that E. coli isolated from women with cystitis were hemolytic 22.5% of the time, while only 10.8% of stool isolates were hemolytic. Using hybridization to an hly gene probe, we found a significant difference in the presence of hly between various UTI (31–48%) and fecal (15%) isolates [13]. CNF1 is also found more often in UTI strains than in fecal strains [13]. Caprioli et al. [14] examined 91 UTI isolates and determined that 37% produced both CNF1 and hemolysin, while among 114 isolates from normal stools only 1 (0.9) produced CNF1. E. coli cnf1 mutants compared to their isogenic $cnf1^+$ parents have decreased virulence in a mouse model of ascending UTI [15]. Using hybridization to a cnf1 gene probe we found a significant difference in the presence of cnf1 between various UTI (27–41%) and fecal (9%) isolates [13]. Sat, a vacuolating cytotoxin, elicits defined damage to kidney epithelium during upper urinary tract infection [34]. Using a sat gene probe, Guyer et al. [33] found 38 out of 67 (55%) pyelonephritis isolates but only six out of 27 (22%) fecal isolates carried the sat gene.

In addition to the sat gene, two other serine protease autotransporter genes are associated with urinary tract infection isolates of E. coli, picU and vat [31,37,38]. PicU has mucinolytic activity [31], while Vat is a vacuolating autotransporter toxin originally found in avian pathogenic E. coli [37].

Several of the potential virulence factors listed in Table 1 are associated with iron uptake systems. The siderophore aerobactin is synthesized by a set of *iuc* genes and proteins encoded by *iut* genes mediate its transport. When we used an *iucD* gene probe, we found the prevalence in our cystitis collections varied between 33% and 46%, which was not statistically different from the 41% found in our fecal isolates [13]. However, *iucD* occurred in a statistically significantly larger percentage (69%) of our pyelonephritis isolates [13]. Further, Torres et al. [29] reported that an *iutA* mutant of CFT073 is out competed by CFT073 in a mouse model of ascending urinary tract infection. A screen of genes upregulated when the UPEC strain CP9 was grown in urine identified the *iroN*$_{E.\ coli}$, which encodes a siderophore receptor, as a gene whose expression increased 27-fold [28] The *iroN*$_{E.\ coli}$ gene occurs consistently more frequently in both cystitis and pyelonephritis isolates compared to fecal isolates [13,25,27]. Three additional genes with homology to iron uptake systems have been reported by Rasko et al. [39] to be present more often in *E. coli* cystitis or pyelonephritis isolates compared to fecal ones, and Parham et al. [40] have shown that these *fbp* genes are more common in prostatitis isolates than in cystitis or pyelonephritis isolates.

The genes for group II capsule formation (*kpsMT*) and outer membrane protein T (*ompT*), which is a protease, are fairly common in fecal *E. coli*, but are still statistically significantly more often in UPEC isolates [13,25]. Two bacteriocin genes, *usp* [36] and *cvaC* [16], have been implicated as potential UPEC virulence genes. The evidence is strongest for *usp*, which is most prevalent in pyelonephritis isolates, but is also present in cystitis isolates at a higher frequency than in fecal isolates [25,27]. Currently, the evidence for *cvaC* is only suggestive, as *cvaC* was found in 15.3% of pyelonephritis isolates, 9.1% of cystitis isolates, and 6.8% of asymptomatic bacteriuria isolates; the sample size was too small for these differences to be statistically significant.

Finally, two additional open reading frames present in pathogenicity island II of CFT073 with similarities to transposase genes are more common in UTI isolates than in fecal isolates [39], and this same pathogenicity island II has three genetic loci statistically significantly more often in prostates than in pyelonephritis [40].

3. Are there distinct UPEC pathotypes?

Individual genes or even clusters of genes encoding a single complete virulence factor are not by themselves sufficient to allow bacteria to cause disease. Rather, complementary sets of virulence factors work together to direct bacteria through a particular interaction with the host that can result in disease. Strains of a given bacterial species that have a particular set of virulence factors in common that direct them through a particular pathogenesis process are called a "pathotype." As mentioned earlier, *E. coli* causing enteric/diarrhoeal disease can be grouped into at least six different pathotypes. What is the evidence that *E. coli* causing UTIs may also be made up of distinct pathotypes?

Previous studies by our group demonstrate that *E. coli* causing first UTI have many different constellations of virulence factors. Among 216 *E. coli* causing first UTIs, grouping by the presence of nine known uropathogenic factors led to 36 distinct groups; using Southern analysis, these 36 groups could be divided into at least 125 different strains [45]. These different constellations predict different risks of acquiring a second UTI in the following 6 months [41]. Further, certain O serogroups are found more frequently in UTI than in fecal isolates, but a wide array of O serotypes are found among UTI strains [46]. While this diversity may suggest that any *E. coli* can cause UTI, this is not the case: UPEC can be differentiated from rectal *E. coli* based on a number of parameters including the presence of known virulence factors. Based on these observations, we hypothesize that UTI has multiple molecular pathways that may contribute to pathogenesis.

We screened our *E. coli* isolates from first UTI, second UTI, recurring UTI, UTI in women aged 40–65 years, pyelonephritis in young children, periurethral, and rectal isolates for the presence or absence of 10 virulence genes [13], and created a binary score or "virulence signature". Excluding type 1 pili, which are present in virtually all *E. coli* isolates in our collection, there are 512 possible virulence signatures. However, we observed six virulence signatures that contain over one half of all strains from each collection (UTI case, urinary, vaginal, rectal, etc.), with the distribution of these signatures varying by whether the isolates are urinary, periurethral, or rectal flora. In addition, a common virulence signature from one source (i.e., first UTI) is often found at much lower levels in isolates from another source (i.e., rectal). This conforms to the above model of diverse pathotypes of *E. coli* within the clinical syndrome we call UTI.

Using our previously published data on presence or absence of 10 virulence genes [13], we examined the co-occurrence of these virulence genes among 762 of the isolates. As several gene combinations occurred together more frequently than predicted by chance alone, we decided to classify isolates based on the association between genes. We examined the associations between all genes tested by collection, and defined those with the strongest associations combined with known biologic evidence for physical linkage as a pathotype. The associations between all genes were retested excluding the already identified pathotype isolates. This process was repeated until no obvious groupings remained. Table 2 illustrates an example of this process. A strong

Table 2
Sample pairwise associations used to identify potential uropathogenic *E. coli* (UPEC) pathotypes

	Virulence gene pairs	Value calculated	First, UTI 18–39 years (n = 237)	Second, UTI 18–39 years (n = 42)	Recurring UTI 18–39 years (n = 27)	UTI 40–65 years (n = 134)	Periurethral 18–39 years (n = 53)	Fecal *E. coli* 18–39 years (n = 269)
All Isolates	*cnf1* and *hly*	Odds ratio	>359	>348	>154	>194	>507	423
		P-value	≪1×10^{-16}	9.3×10^{-10}	2.6×10^{-10}	≪1×10^{-16}	4.3×10^{-12}	≪1×10^{-16}
	hly and *papG*$_{AD/IA2}$	Odds ratio	0.91	1.1	1.1	1.3	0.52	1.8
		P-value	0.755	0.892	0.835	0.063	0.565	0.124
Isolates remaining after removal of pathotype 1	*cnf1* and *hly*	Odds ratio	>91.1	>116	>42	>41.2	>78	114
		P-value	4.3×10^{-16}	2.9×10^{-7}	8.0×10^{-6}	7.6×10^{-8}	1.7×10^{-7}	2.2×10^{-16}
	hly and *papG*$_{AD/IA2}$	Odds ratio	2.9	4.2	2.7	12.8	3.4	4.9
		P-value	0.003	0.156	0.182	2.6×10^{-7}	0.328	2.6×10^{-4}
Isolates remaining after removal of pathotypes 1 and 2	*cnf1* and *hly*	Odds ratio	>5.7	ND	ND	ND	ND	16.3
		P-value	0.019	(no *cnf1*)	(no *cnf1*)	(no *cnf1*)	(no *cnf1*)	0.01
	hly and *papG*$_{AD/IA2}$	Odds ratio	6.2	>6.3	>7.2	27.5	>6.8	7.3
		P-value	1.7×10^{-5}	0.023	0.015	2.0×10^{-7}	0.016	7.3×10^{-5}

association was identified between *cnf1* and *hly* in all collections, consistent with their known joint mapping to the PAI V pathogenicity island in J96 [47]. PAI V also contains the *prsG*$_{J96}$ gene. Because of this association with a potentially important UTI PAI, we define strains that are *cnf1*$^+$*hly*$^+$*prsG*$_{J96}^+$ as pathotype 1.

After removing pathotype 1 strains from the analysis, *cnf1* and *hly* are still strongly associated with each other (Table 2), and with *sfa* as well (data not shown). Further, *hly* also appears to be associated with *papG*$_{AD/IA2}$, an association that does not exist when all isolates are compared (Table 2). Therefore, we defined all strains that were *cnf1*$^+$*hly*$^+$*sfa*$^+$, after the removal of pathotype 1 strains, as pathotype 2 strains. The strains containing *cnf1* are almost always grouped as either pathotype 1 or pathotype 2. When the pairwise association analysis was performed on the remaining strains after removal of both pathotype 1 and 2 strains, *hly* was positively associated with *papG*$_{AD/IA2}$ at a level that reaches statistical significance in each of the collections (Table 2).

We sequentially defined a total of five pathotypes using this strategy (Table 3). The groups are hierarchical: pathotype 1 takes precedence over pathotype 2 and so forth. Pathotypes 1–4 occur in at least two of the UTI collections at two or more times the frequency with which they are found in fecal isolates. This was expected based on the UTI specificity of the individual genes used to define them. In addition, the distribution of pathotype strains among the different collections differs from that expected by chance alone ($p = 2.0\times10^{-10}$).

Four of the five pathotypes occurred more frequently in the UTI than fecal isolates. These first four pathotypes account for most of the previously described urinary *E. coli* virulence genes. Pathotype 5 differs in several important respects. It is defined by the presence of two commonly occurring genes, *kpsMT* and *ompT*, in the strains remaining after the four UTI enriched pathotype strains are removed from analysis. Pathotype 5 strains made up about 37% of all isolates we analyzed, and occurred in a slightly higher percentage of fecal than UTI isolates. We hypothesize that pathotype 5 strains are a combination of different pathotypes, some predominantly UTI and others mainly fecal, that we cannot distinguish using current probes. To break pathotype 5 into UTI vs. fecal pathotypes we need additional *E. coli* genes found primarily in either UTI or fecal pathotype 5 isolates.

In women aged 18–39 with first UTI, there were no statistically significant differences in bacterial count, hematuria, specific gravity or pH by pathotype. However, pyuria and the self-reported symptoms of back pain and chills occurred more often in women with first UTI infected with *E. coli* pathotype 3 (*aer*$^+$, *hly*$^+$, *papG*$_{AD/IA2}^+$). There was also a statistically significant ($p = 0.03$) difference between the pathotype distribution

Table 3
Distribution of proposed uropathogenic *E. coli* (UPEC) pathotypes by isolate collection

Pathotype (genotype)	First, UTI 18–39 years (n = 237)	Second, UTI 18–39 years (n = 42)	Recurring UTI 18–39 years (n = 27)	UTI 40–65 years (n = 134)	Peri-urethral 18–39 years (n = 53)	Fecal 18–39 years (n = 269)	Total (n = 762)
Pathotype 1 (*cnf1, hly, prsG_{J96}*)	47 (19.8%)	8 (19.1%)	8 (29.6%)	37 (27.6%)	11 (20.8%)	19 (7.1%)	138 (17.5%)
Pathotype 2 (*cnf1, hly, sfa*)	15 (6.3%)	4 (9.5%)	3 (11.1%)	10 (7.5%)	2 (3.8%)	6 (2.2%)	43 (5.4%)
Pathotype 3 (*aer, hly, papG_{AD/1A2}*)	19 (8.0%)	1 (2.4%)	2 (7.4%)	13 (9.7%)	1 (1.9%)	10 (3.7%)	48 (6.1%)
Pathotype 4 (*aer, kpsMT, ompT, drb*)	22 (9.3%)	4 (9.5%)	1 (3.7%)	4 (3.0%)	2 (3.8%)	9 (3.4%)	43 (5.4%)
Pathotype 5 (*kpsMT, ompT*)	86 (36.3%)	16 (38.1%)	8 (29.6%)	42 (31.3%)	22 (41.5%)	109 (40.5%)	291 (36.9%)
All remaining strains	48 (20.3%)	9 (21.4%)	5 (18.5%)	28 (20.9%)	15 (28.3%)	116 (43.1%)	226 (28.6%)

found in white versus black women with first UTI, with pathotypes 1, 3 and 4 being more common in isolates from black women.

While we feel existing evidence is good that separate UPEC pathotypes exist, it is very clear that we have a very limited current understanding of the critical genetic differences that truly define the different pathotypes. Equally importantly, use of current known virulence factors to create potential pathotypes has not yet given the resolution needed to match specific potential pathotypes with clinical characteristics of disease. Discovery of additional *E. coli* genes involved in uropathogenesis and determination of their distribution and co-occurrences will help further define UPEC pathotypes and allow for a more detailed analysis of how these pathotypes might differ in how they cause disease.

4. Concluding remarks

Being able to identify particular pathotypes of a bacterial species has proved very helpful in understanding the particular disease processes involved and in identifying particular virulence factors for therapeutic or vaccine development. Clearly, we need to determine the distributions of many potential UPEC virulence genes, both those listed in Table 1 and others as they are discovered, in large collections of epidemiologically well defined collections of both fecal and rectal *E. coli* compared to the various categories of UTI isolates. This process will be facilitated by the existence of a complete genome sequence for the pyelonephritis strain CFT073 [48], and by using high throughput screen methods such as the Library on a Slide approach we have recently published [49].

Acknowledgements

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