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Combating enteropathogenic *Escherichia coli* (EPEC) infections: the way forward

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Abstract

Enteropathogenic *Escherichia coli* (EPEC) strains continue to cause severe and sometimes fatal infantile diarrhea, particularly in Africa. Increased efforts at diagnosis, defining the clinical spectrum of disease, understanding pathogenic mechanisms, and delineating immune responses are desperately needed to develop new strategies to combat EPEC.

Keywords

translational research; diarrhea; mortality; infants; immunity; vaccines

Introduction

The recently completed Global Enteric Multicenter Study (GEMS) was a prospective, population-based case control study involving seven sites in Africa and Asia with the goal of identifying the etiology, burden, and associated mortality related to acute moderate-to-severe diarrhea in children less than 5 years of age [1]. One of the many findings to emerge from GEMS was the association of typical strains of EPEC with mortality, particularly in infants in Africa [2]. The serious outcomes that continue to be associated with EPEC demand a reassessment of current strategies used to understand, treat, and prevent infections due to this pathogen. Despite remarkable progress in unraveling the molecular pathogenesis and cell biology of EPEC infection, there has been little translation of this knowledge into clinical practice. This gap was the subject of a recent International Workshop on EPEC. The goal of this article is to delineate the challenges and potential solutions that help define a path forward, as discussed in that workshop (Box 1).

Challenge 1: methods to define and diagnose EPEC infections are not universally accepted

A full accounting of the health burden posed by EPEC infections cannot be made without agreement on the definition and diagnosis of EPEC. At the second international meeting on

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EPEC in Saõ Paulo, Brazil in 1995, EPEC were defined as 'diarrheagenic *E. coli* that produce a characteristic histopathology known as attaching and effacing (A/E) on intestinal cells and that do not produce Shiga, Shiga-like or Verocytotoxins. Typical EPEC (tEPEC) of human origin possess a virulence plasmid known as the EAF (EPEC Adherence Factor) plasmid that encodes localized adherence on cultured epithelial cells mediated by the Bundle Forming Pilus (BFP), while atypical EPEC (aEPEC) do not possess this plasmid. The majority of typical EPEC strains fall into well-recognized O:H serotypes' [3]. With the advent of new diagnostic techniques, increasing availability of complete genomic sequencing, and better appreciation of *E. coli* phylogeny, the validity of this definition deserves reappraisal. Diagnostic techniques such as single-target, multiplex, and quantitative PCR assays have become more widely adopted. In agreement with the 1995 definition, the generally accepted targets of these assays for EPEC diagnosis include the Shiga toxin genes *stx1* and *stx2*, which must be absent; *eae*, which encodes intimin and which must be present; and *bfpA*, which is present in typical but not atypical strains (Table 1). However, there are both general concerns regarding the EPEC definition and specific concerns regarding the PCR targets.

Some epidemiological studies have suggested that the current definition does not optimally distinguish between EPEC strains that cause acute diarrhea and strains that do not. For example, in some case control studies only the subgroup of EPEC strains that belong to classic serotypes is significantly associated with diarrhea, whereas those that do not belong to such serotypes are cultured from cases and controls with equal frequency [4]. Furthermore, in aEPEC, the presence of certain additional genomic markers is strongly associated with chronic diarrhea [5].

Further studies are needed to determine whether the inclusion of additional information, including the presence or absence of certain genes, improves the diagnostic value of tests for EPEC. Such studies require accurate data regarding clinical variables of interest and should include strains from diverse geographical sources. Ideally, these clinical data will not be limited to the presence or absence of diarrhea, but will encompass measures of overall health before, during, and after the sample is collected. When these clinical measures are integrated with whole genome sequence data, it is likely that new targets will be identified for inclusion in refined diagnostic tests.

Concerns also arise regarding the application of currently available diagnostic tests. The *eae* and *bfpA* genes have significant allelic variability. Current primers are designed to avoid polymorphic regions of the genes, but it is not known whether some strains have additional unknown variability. Furthermore, examination of these genes alone can be misleading. For example, strains that have the *bfpA* gene, but deletions in other essential genes of the *bfp* operon, would be classified using currently accepted tests as tEPEC, although they behave phenotypically as aEPEC.

To address this, genome sequences must be examined on an ongoing basis to insure that all EPEC strains are correctly assigned. If necessary, the targets should be adjusted accordingly. An internationally recognized standard for EPEC diagnostic testing should be established and refined as necessary.

Challenge 2: our understanding of the prevalence, spectrum of disease, and consequences of EPEC infections is incomplete

The epidemiology of EPEC disease is dynamic and insufficiently described. EPEC strains were first identified in the mid-20th century as the cause of devastating outbreaks of neonatal diarrhea principally in more economically privileged countries [6]. As these outbreaks became less common in the latter half of the century, case control studies performed over six continents demonstrated that EPEC remained a leading cause of infant diarrhea in less economically advantaged countries [7]. The association with diarrhea was particularly strong in infants less than 6 months of age infected with tEPEC strains. In the past decade, case control studies from selected countries have identified EPEC as an important cause of pediatric diarrhea [8]. However, it is apparent that the epidemiology of EPEC infection has shifted. In countries throughout South America where the prevalence of EPEC infection had been high, recent studies have not identified a significant association between EPEC and infant diarrhea. Meanwhile, the proportion of aEPEC strains has increased in both cases and controls to the point where aEPEC strains often outnumber tEPEC strains [9] and aEPEC strains have also been associated with childhood diarrhea in economically advantaged countries [10–12]. However, these studies suggesting a declining importance of tEPEC were not performed where the burden of diarrhea is highest.

The recently completed GEMS shed important new light on the current epidemiology and clinical consequences of EPEC infection. GEMS is the largest study of the etiology of acute moderate-to-severe childhood diarrhea ever conducted, with over 40 bacterial, viral, and parasitic microbes sought using standardized methods [1]. At most GEMS study sites, tEPEC strains were not among the leading pathogens to which the risk of acute moderate and severe diarrhea could be attributed. Strikingly, however, tEPEC infection was associated with a 2.8-fold elevated risk of death among infants, the greatest pathogen-attributable risk for death observed [2].

Thus, recent studies of the epidemiology of EPEC infection have painted a complex picture of the global burden of this pathogen. In many countries, tEPEC strains no longer seem to be a major cause of acute diarrhea. Although aEPEC continue to be isolated worldwide, the association of such strains with acute diarrhea is inconsistent. However, the specter of severe outcomes associated with tEPEC has reemerged.

Additional epidemiological studies of the global burden of tEPEC and aEPEC infections are urgently needed. Such studies must use standardized definitions and diagnostic techniques as described in Challenge 1. Such studies must include relevant clinical correlates and follow-up to include acute diarrhea, chronic diarrhea, growth stunting, and mortality. Nested environmental and contact studies would provide needed data to assess transmission.

Challenge 3: a thorough understanding of the mechanisms leading to EPEC disease in humans has yet to be achieved

Great strides in unraveling the molecular pathogenesis of EPEC infection have been made [13]. However, a comprehensive understanding of the pathogenicity mechanisms relating to

disease remains elusive. Without such an understanding, it is difficult to develop novel strategies for intervention. Poorly understood aspects of EPEC infection include transmission dynamics, reservoirs, the precise mechanisms leading to diarrhea, the mechanisms responsible for these consequences of infection other than diarrhea, the relationship between attaching and effacing and disease, and factors associated with protection from infection and its consequences. Arguably the foremost obstacle to achieving this understanding is the lack of a robust animal model. The natural murine pathogen *Citrobacter rodentium* displays many of the characteristics of human EPEC strains, but differences between this pathogen and EPEC, and between mice and humans cast doubt on this model. Although aEPEC can be isolated from many different species, and tEPEC from a few, such strains are distinct from those that cause disease in humans. Furthermore, with the exception of infant macaques [14], strains isolated from humans do not cause diarrhea in animal models.

Further studies on pathogenesis should be directed at clinically relevant questions and translational knowledge involving transmission, mechanisms of diarrhea, the role of the microbiome, and other host factors. Additional efforts toward improving animal models should be pursued.

Challenge 4: our knowledge of the role of immunity in protection against EPEC infections is woefully inadequate

Multiple studies have confirmed that EPEC strains, particularly tEPEC strains, are most closely associated with acute diarrhea in the very youngest infants. However, whether this association is due to inherent susceptibility that declines with age or to acquired immunity has never been determined. In fact, despite many studies investigating EPEC antigens, protective immunity has never been convincingly demonstrated. This knowledge is critical when considering a potential vaccine. In one study a lower attack rate in adult volunteers rechallenged with tEPEC compared to naïve volunteers was observed, but that study was underpowered to determine significance [15].

To address this, adequately powered studies should be conducted in volunteers or nonhuman primates to determine whether infection with EPEC confers protective immunity. Correlates of protection should be sought including humoral and cell-mediated responses to target EPEC antigens. Consideration should also be given to studying the effect of passive immunization with hyperimmune bovine milk concentrate from cows immunized with relevant antigens in a population that has a high burden of EPEC disease. A passive immunization study could provide the proof of principal studies required to assess the feasibility of an EPEC vaccine.

Concluding remarks

EPEC, once feared in the UK and US, continues to be associated with severe outcomes in areas of the world where the burden of diarrhea is highest. Further research should be guided by the goal of eliminating this menace.

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Box 1. Specific recommendations for progress against EPEC infections

- The definition and diagnosis of EPEC infection should be standardized.
- There should be additional epidemiological study of the consequences of EPEC infection.
- Translational studies of EPEC pathogenesis should be performed.
- Protective immunity against EPEC should be evaluated.
- An intervention study should be performed in a carefully selected population.

Table 1

Currently accepted gene targets for the identification of EPEC and related bacteria

Designation	**Abbreviation**	**Gene presence or absence**[a]		
		eae	*bfpA*	*stx*
Attaching and effacing *E. coli*	AEEC	+	+/−	+/−
Enteropathogenic *E. coli*	EPEC	+	+/−	−
Typical EPEC	tEPEC	+	+	−
Atypical EPEC	aEPEC	+	−	−

[a] Symbols: +, indicates presence; −, indicates absence.