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The potential of using *E. coli* as an indicator for the surveillance of antimicrobial resistance (AMR) in the environment☆

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To understand the dynamics of antimicrobial resistance (AMR), in a One-Health perspective, surveillance play an important role. Monitoring systems already exist in the human health and livestock sectors, but there are no environmental monitoring programs. Therefore there is an urgent need to initiate environmental AMR monitoring programs nationally and globally, which will complement existing systems in different sectors. However, environmental programs should not only identify anthropogenic influences and levels of AMR, but they should also allow for identification of transmissions to and from human and animal populations. In the current review we therefore propose using antimicrobial resistant *Escherichia coli* as indicators for monitoring occurrence and levels of AMR in the environment, including wildlife.

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Introduction

To manage the threat of Antimicrobial Resistant (AMR) bacteria in animal and human health a harmonized,

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multisectoral 'One-Health', surveillance of AMR is needed [1]. However, current surveillance programs primarily focus on AMR in livestock and isolates from human clinical cases, with environmental perspectives, including wildlife, generally omitted. That there is a need for a comprehensive international environmental AMR monitoring has also been highlighted by the Codex Alimentarius Intergovernmental Task Force on Antimicrobial Resistance (FAO, http://www.fao.org/fao-who-codexalimentarius/committees/committee/en/?committee=TFAMR). Several recent reviews have also addressed this need and has made suggestions on objectives and approaches for such a surveillance [2,3••,4]. In this review, we will focus on the potential of *Escherichia coli* being an indicator for monitoring occurrence of clinically important antibiotic resistant bacterial phenotypes, such as carbapenems, colistin, and extended spectrum beta-lactams (ESBL) in the environment. We will address, how *E. coli* is used: (i) as an indicator in AMR surveillance systems worldwide; (ii) for anthropogenic faecal pollution of surface water; (iii) in studies that have described multi-drug resistant *E. coli* in the environment; and (iv) availability of standardized laboratory protocols for handling *E. coli*.

Goals of environmental AMR surveillance

An indicator for surveillance of AMR in the environment should not only be suitable for reporting environmental AMR levels and how these are influenced by anthropogenic activities, but it should also enable estimating the potential risk of transmission to and from human and animal populations. Since surveillance systems already exist both in human health and livestock sectors, an environmental indicator should also complement these efforts. Another purpose of environmental surveillance could be to inform about circulation of AMR in the human population, thus improving current human clinical surveillance systems.

E. coli in current AMR surveillance programs

E. coli is implemented in a multitude of national surveillance programs with several programs producing integrated national reports with human clinical data, livestock carriage and occurrence on meat-products, with some reports also including and clinical veterinary data. Examples of integrated European national reports are Swedres-Svarm, RESAPATH, UK One Health report, NethMap and Danmap [5–9]. Similar reports are also produced in the United states (NARMS, https://www.cdc.gov/narms) and Canada (CIPARS) [10]. However, to our knowledge only the Norwegian program NORM has recommended including environmental perspectives, but there are some infrequent reports on wildlife from surveillance activities [11–14]. Supranational AMR surveillance programs also exist (Table 1) with the largest strategy being the WHO:

Table 1

Surveillance systems including *E. coli* standardized by supranational organisations (i.e. national schemes are not included but existing)

	Epidemiological unit	Sample type	Nonselective isolation	Selective isolation	Numbers/year	AMR testing
WHO	GLASS EUCAST or CLSI AST testing	Patient	Clinical sample (e.g. blood, urine.)	Yes	No	Depending on numbers of isolates collected
WHO	tricycle EUCAST or CLSI AST testing	Patient, person, farm, surface water location	Clinical, healthy humans, animals, surface water	No	ESBL	In total about 300/year
ECDC	Patient	Clinical sample (e.g. blood, urine, Cerebral spinal fluid)	Yes	Yes	Depending on numbers of isolates collected	EUCAST AST testing
EFSA	Herds Meat product	Lifestock (cecal samples at slaughter) Retail products	 Yes	ESBL, pAmpC CPE (starting 2021)	170 *E. coli*/year/country and ESBL positive *E. coli* isolates from 170 samples/year/country	EUCAST AST testing
EARS-Net	Patients	Clinical sample (e.g. blood, urine,)	Yes	No	Depending on numbers of isolates collected	EUCAST AST testing
EARS-Vet[a]	Diseased Animals or Herds	Clinical samples from cattle, swine, chickens (broiler and laying hen), turkeys, cats and dogs	Yes	No	Depending on numbers of isolates collected	EUCAST AST testing

[a] Not fully implemented during the preparation of this article.

The Global Antimicrobial Resistance and Use Surveillance System (GLASS) established in 2015, incorporating 92 countries across diverse income levels (https://www.who.int/initiatives/glass/). GLASS provides standardized protocols to capture the frequency of resistance among high-priority pathogens including *E. coli* from bloodstream or urinary tract infections. With respect to One-Health surveillance WHO has brought forward the Tricycle Protocol, covering monitoring of ESBL-producing *E. coli* in humans, animals, and the environment, which has been piloted in six countries and is currently being rolled out in additional countries (https://www.who.int/initiatives/glass/glass-modules-7). In Europe, the European Antimicrobial Resistance Surveillance Network (EARS-Net) coordinated by the European Centre for Disease Prevention and Control collects, on a voluntary basis, clinical AMR data from local laboratories, including data on *E. coli* from blood and cerebrospinal fluid (https://www.ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/ears-net). In comparison monitoring of AMR *E. coli* from livestock and retail meat samples are mandatory within the European union [15]. This monitoring is harmonized by the European Food Safety Authority (EFSA) and includes determining resistance profiles of commensal *E. coli* isolates from unselective screening, as well as selective screening for ESBL-producing and AmpC producing *E. coli*, and carbapenem-resistant *E. coli* [16]. Several European agencies also suggested that an EARS-Net in veterinary medicine should be established and integrated with the other monitoring systems. With the current EARS-Vet this development is on its way [17••,59].

E. coli as an indicator of anthropogenic impact on the environment

E. coli has long been a water quality indicator in the EU Bathing Water Directive and is currently one of the parameters to classify the quality of bathing waters, based on systematic monitoring of *E. coli* throughout the recreational season [18,19]. Similarly, *E. coli* are included in the WHO guidance on recreational water for its specificity as an indicator of faecal pollution from humans and warm-blooded animals [20,21]. The reason for using *E. coli* as an indicator is that it appears only at low background levels in the environment but has high survival rates [22–24]. It is also interesting to note that recent studies have shown that AMR profiles of *E. coli* isolates from sewage samples correlate to the *E. coli* AMR data from the associated populations [25,26,27••]. AMR *E. coli* has also been extensively described in different environmental departments including ESBL-producing and carbapenemase-producing *E. coli* from wildlife and surface waters [28•,29,30•]. Interestingly *E. coli* diversity appears to be higher in surface waters compared to wastewaters, but with AMR levels being higher in wastewater [27••].

Available methodology for *E. coli*

There are several ISO-standard methods for quantification of *E. coli* in water based on membrane filtration or most probable number techniques, but there are no standardized methods for the quantification in other environmental matrices, such as soils and sediments, or for AMR *E. coli* [31,32]. However, standardized protocols for selective cultivation of AMR *E. coli* from human and animal samples could be easily adapted to environmental monitoring [33]. Culture-based methods for quantifying and isolating *E. coli* are comparatively inexpensive and simple to employ, ensuring their applicability across high and low-income countries (LMIC) that vary widely in laboratory capacity and technical capability.

With respect to antibiotic sensitivity testing (AST) of *E. coli* isolated from environmental samples, necessary data and methods for AST are available through https://clsi.org/ and https://eucast.org. For example, the EFSA AMR monitoring protocol recommends the use of broth microdilution, provides a list of antibiotics to be tested, and uses the EUCAST epidemiological cut-off values (ECOFFs) [34]. However, AST is limited in that the underlying mechanism of resistance remains unknown, but PCR and sequencings protocols for specifically relevant genes and mutations are available.

Methods for characterization of *E. coli* are also available, making it easy to compare environmental *E. coli* isolates to human and animal isolates. For example, serotyping using O-antigens and H-antigens has been a gold standard for subtyping *E. coli* for epidemiological activities for decades but has today largely been replaced with molecular-based methods primarily multi-locus sequence type (MLST) due to greater accuracy (https://pubmlst.org/organisms). MLST is based on variations in seven house-keeping genes and a large public MLST database exists (https://enterobase.warwick.ac.uk/). *E. coli* range from being a commensal to well-known pathogens, with their Sequence Types (STs) reflecting this diversity. Some STs, such as ST131, ST95 are associated with human disease but are rarely detected in other compartments/environments [57]. In contrast other STs, such as ST10, are ubiquitous and have been reported from human infections, animals and the environment [58]. With the expansion of whole-genome-sequencing (WGS) almost the complete genome or only the core genome, for example, genes present in all isolates, can now also be used to define *E. coli* subtypes and a defined core genome (cg) MLST scheme is already available in Enterobase [35]. WGS provides a more accurate subtyping, due to significant variability in *E. coli* genomes, but it is generally more time and cost consuming and needs bioinformatics expertise. The cost and analyze requirements might also limit implementation by some LMICs. It is currently proposed that WGS be incorporated into

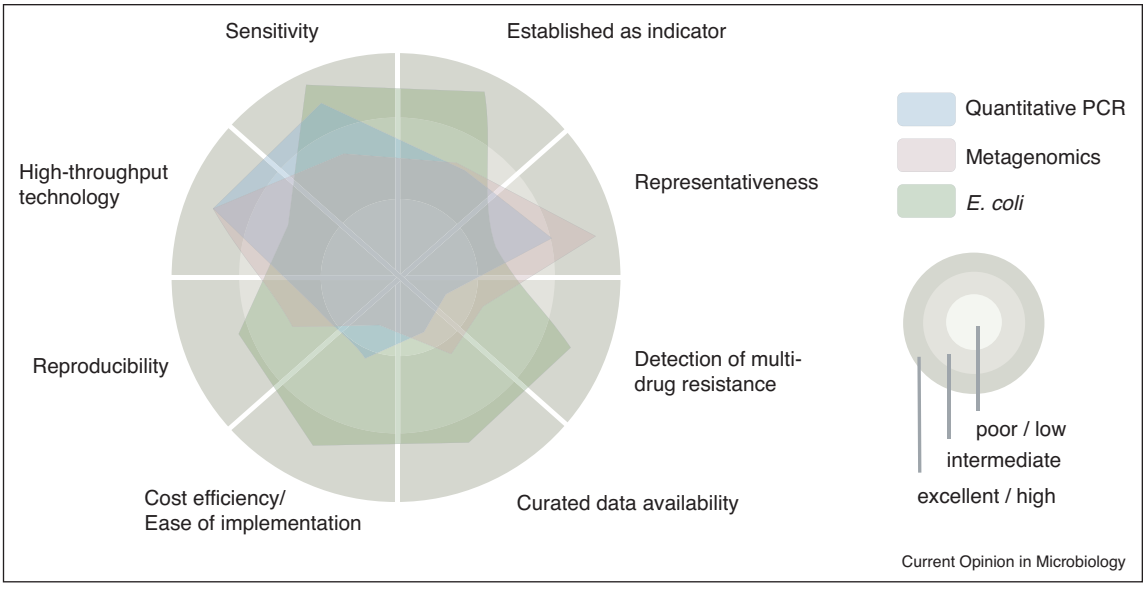
EFSA monitoring by 2026, and GLASS is also preparing WGS guidance documents [16,36].

In contrast to phenotyping and older molecular methods, WGS yields far more information including the identification of AMR genes (that are currently known), their genetic context including co-linkage and association with plasmids and other mobile genetic elements, and the phylogenetic relationships between isolates [37]. For example, traditional methods can only test for a handful antibiotics at once while WGS data can be screened for all known genetic determinants of AMR at once. Open-access databases and software are already available for this type of screening, and studies comparing *E. coli* WGS genotyping, and phenotyping have shown good correlations [38,39,40•]. WGS also enables detection of clones and transmission of AMR plasmids, for example multi-drug-resistant *E. coli O25:H4*-ST131 has been associated with an ongoing global human pandemic and has been shown to occur in the environment and animals [41–44]. Another important factor in using WGS to characterize *E. coli* isolates is in the description and tracing of new genes for example the *mcr* genes which confers resistance to the last resort antibiotic colistin. After the first description of *mcr*-1 in humans and livestock in 2015 in China, WGS and available genome databases revealed the rapid global expansion of the plasmid-borne gene in *E. coli* strains and other hosts carried in food, domesticated animals, wildlife, and various environmental compartments [45,46]. To date 10 different *mcr* genes, all of them identified with the help of WGS, have been reported.

Limitations of using *E. coli* for environmental surveillance

E. coli as an indicator species mainly provides a snapshot of the environmental dimension of the faecal transmission route. The evolutionary processes underlying the spread and risk of AMR in the environment, with processes such as novel resistance mechanisms, selection and mobilization of pre-existing resistance determinants and horizontal gene transfer are difficult to track using just *E. coli*. A draw-back of using selective culture-based monitoring is that differences exist in the methodology used at different compartments making it difficult to compare between compartments/countries [47]. Therefore, there is a need to evaluate protocols for environmental monitoring and deciding on quality controls measures. A limitation of using culture is that throughputs generally are low, which is not an extensive limitation when monitoring AMR *E. coli* in infections, as usually only one pathogenic *E. coli* strain is predominant. However, in the environment where a multitude of diverse *E. coli* with different properties are present, proper sensitivity will be difficult to capture when focusing on randomly collected *E. coli* [27••,48]. In addition, there is a risk that only the most abundant and prolific strains will be detected, exceeding non-cultivatable or difficult to cultivate strains [24]. These problems are also shared with AMR monitoring

Figure 1



Advantages of using *E. coli* as an indicator for AMR in the environment. Different features are given which may be considered to measure its usefulness in comparison to other indicators such as using quantitative PCR and metagenomics that can evaluate AMR in the environment. The different colours represent the different methods and the different grey shades indicate the suitability of the methods ranging from poor/low to excellent/high.

of faecal carriage as several *E. coli* strains are simultaneously carried in human and animal guts [49]. The sensitivity of detecting *E. coli* while however increase when using selective cultivation for specific AMR phenotypes [51]. Different *E. coli* can also vary in environmental fitness due to a variety of attributes and site-specific circumstances, and some *E. coli* have their own life cycles in the environment and naturalized strains exist [24,52••]. Occurrence of *E. coli* in the environment might also be impacted by faecal pollution from wildlife [53,54]. Thus, at least in some environments, differentiation between direct anthropogenic impact and 'natural' populations might be difficult to achieve when relying on phenotype. Consequently, a need may exist to include additional indicators of faecal pollution, for example, crAssphage and *Bacteroidales*, to support interpretations of *E. coli* based monitoring data [55••,56]. *E. coli* is also not a suitable indicator of AMR in the natural microbiota, where microbiome studies might be more appropriate.

Conclusions

There is an urgent need to implement AMR environmental surveillance, and *E. coli* could be used as an indicator both for specific resistance phenotypes as well as more broadly looking at randomized isolates, thus complementing surveillance in humans and livestock. Using *E. coli* as an indicator for levels and anthropogenic influences of AMR in the environment has some key advantages compared to other methods (Figure 1): (i) comparisons to data from human and animal sectors are possible; (ii) analysis are relatively cost-effective; (iii) is easy to implement; (iv) protocols are available; (v) it is an established indicator of anthropogenic influences in the environment; (vi) and currently cultivation based methods are the best method for detection and in-depth analysis, including tracking transmission, of AMR *E. coli*. To provide a deeper insight into AMR circulating in the total bacterial community alternatives exists in metagenomics or different qPCR techniques (Figure 1). However, harmonized protocols and bioinformatic tools are not readily available, the sensitivity, specificity and reproducibility of the methods needs to be improved, also it is currently not possible to use for detecting AMR plasmids, and it is not feasible to implement globally, especially in LMICs, in the near future. In contrast national references laboratories already have the capability of implementing AMR *E. coli* culture and can readily extend it to environmental samples.

Author contribution

The authors contributed equally to the conceptualization and writing of the review and the order of the authors has only technical reasons.

Conflict of interest statement

Nothing declared.

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