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Short communication

Classification and ranking of shigatoxin-producing *Escherichia coli* (STEC) genotypes detected in food based on potential public health impact using clinical data

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ABSTRACT

Risk classification and management of shigatoxin-producing *E. coli* (STEC) isolated from food has been hampered by gaps in knowledge about the properties that determine the extent to which different subtypes of STEC can cause severe disease. Data on the proportion of infected human cases being affected by severe illness enables an evaluation of existing approaches for classifying STEC strains and the development of a new public health based approach. Evaluations show that existing approaches do not unequivocally classify different STEC variants according to their ability to cause severe disease. A new approach for ranking of STEC genotypes, combining the estimated probability of the strain to cause severe illness with the public health burden associated with the illness in terms of DALY per case, address these limitations. The result is a list of STEC genotypes in descending order of potential public health burden per case. The approach is risk based in considering the probability and consequences following infection (severe illness), and can support transparent risk management. This is illustrated by, arbitrarily, separating the ranked list of genotypes into classes based on the potential public health burden, and by characterising collections of strains isolated from different foods into different classes. Further, the classification of food samples as satisfactory or not based on the cost in terms of proportion of food being rejected and the benefit in terms of the proportion of strains causing severe illness (HUS) that are being captured is demonstrated using this approach.

1. Introduction

Risk classification of shigatoxin-producing *E. coli* (STEC) isolated from food has been hampered by gaps in knowledge about the properties that determine the extent to which different subtypes of STEC can cause severe disease (*e.g.*, NACMCF, 2019). An early approach classified STEC into seropathotypes, based on results from serotyping (Karmali et al., 2003). Later, the European Food Safety Authority (EFSA) proposed a modification of the seropathotype concept that included an alternative molecular approach, utilising genes encoding virulence characteristics additional to the presence of *stx* genes (EFSA, 2013). In 2019, a joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) proposed a concept in which the pathogenic potential of a STEC strain is categorised solely based on virulence gene content (WHO/FAO, 2019). Likewise, based on an analysis of the confirmed reported human STEC infections in the EU/EEA 2012–2017, EFSA (EFSA, 2020) concluded that STEC serogroup cannot be used as a predictor of clinical outcome. EFSA also concluded that any STEC subtypes can be associated with severe illness, but strains with the gene for producing the toxin subtype Stx2a showed the highest rates of haemolytic uraemic syndrome (HUS), hospitalisation and bloody diarrhoea (BD), and presence of the *eae* gene is not essential but was an aggravating factor.

The objectives of this study are to: (i) Compare the JEMRA proposal (WHO/FAO, 2019), for classifying STEC strains detected in food with the probabilities of severe clinical outcomes following infections with the same strains (EFSA, 2020), (ii) Develop and illustrate an approach for the ranking and classification of STEC strains based on potential public health burden, and (iii), Evaluate the new approach by classifying STEC strains detected in food or isolated from human cases, and illustrate the impact of different acceptable limits of potential public health burden in a cost-benefit context.

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Table 1
Comparison between the proposed levels of JEMRA (WHO/FAO, 2019) and the estimated probability to cause severe illness (Table 3, HUS, hospitalisation, BD) according to data in (EFSA, 2020)[1]. Since several STEC genotypes may be included in each risk level (the isolate has more toxin genes than the classification is based on) a range of probabilities from lowest to highest is shown.

		Probabilities according to data in EFSA 2020		
JEMRA level	Virulence genes	HUS	Hospitalisation	Bloody diarrhoea
1	*stx2a* + *eae* or *aggR*	0.208–0.290	0.564–0.593	0.566–0.655
2	*stx2d*	0.103[2]	0.333[2]	0.160[2]
3	*stx2c* + *eae*	0.008–0.043	0.198–0.319	0.239–0.675
4	*stx1a* + *eae*	0.012	0.276	0.273
5	Other sub-types of *stx*	0.000[3]–0.104[4]	0.064[5]–0.320[4]	0.080[6]–0.318[7]

[1] Data for genotypes with less than 20 observations (reported cases) were not included in the report.
[2] Without *eae*, fewer than 20 observations with *eae*.
[3] *stx1a, stx2e* and *stx2g* without *eae*.
[4] *stx2a* without *eae*.
[5] *stx1a* and *stx2b* without *eae*.
[6] *stx1a* without *eae*.
[7] *stx2e* without *eae*.

2. Materials and methods

2.1. JEMRA approach for classification of STEC strains

JEMRA presented a ranking of STEC strains with the presence of various virulence genes (*stx, eae*, and *aggR*) into 5 levels based on their potential to cause diarrhoea, bloody diarrhoea and haemolytic uraemic syndrome (WHO/FAO, 2019). The classification was provided as a guidance to targeted STEC risk management by indicating the relation between different genes and potential health outcomes (Table 1).

2.2. New approach for classifying STEC strains based on potential public health burden

An approach to rank STEC genotypes based on their probabilities to cause severe illness, and the associated public health burden, was developed. Data on the probability to cause severe illness was the proportion of reported STEC cases with the different clinical outcomes haemolytic uraemic syndrome (HUS), hospitalisation, and bloody diarrhoea (BD). Based on a collation of TeSSY data between 2012 and 2017, EFSA summarised such data for each *stx/eae* genotype for which there existed a sufficient number, 20, of reported cases (Fig. 2, in (EFSA, 2020).

The public health burden for each STEC genotype for these clinical outcomes were expressed as disability adjusted life years, DALY (Devleesschauwer et al., 2014). $PPHB_{outcome}$ is the potential public health burden associated with a STEC genotype (DALY per infected case due to the different clinical outcomes HUS, hospitalisation or BD) and calculated as:

$$PPHB_{outcome} = \textit{the probability per infected case to cause the outcome} \times \textit{the public health burden per case with the clinical outcome}$$

STEC genotypes evaluated were ordered in descending order based on the largest potential public health burden outcome, in most cases HUS. This approach was chosen because data were missing for some outcomes and genotypes so summarising over all clinical outcomes would not be appropriate. The ordered list of STEC genotypes may then be grouped into different classes by selecting appropriate limits for potential public health burden based on risk management considerations.

2.3. Evaluation of the new approach by classification of food and human STEC strains

The new approach was evaluated by ranking and classification of STEC food strains collected during four different surveys conducted by the SFA and one outbreak strain isolated from beef: non-domestic beef (Livsmedelsverket, 2014), unpasteurised milk from Swedish farms (Flink and Nyberg, 2020), domestic beef (Livsmedelsverket, 2016), and domestic and non-domestic lamb meat (Livsmedelsverket, 2019). In total, 184 STEC isolates with complete characterisation (*stx-subtype* and *eae*) are included, of which 19 isolates from beef, 25 from unpasteurised milk and 120 from lamb meat could be classified. Results from the surveillance of EHEC (Enterohemorrhagic *E. coli*) are reported by the Public Health Agency of Sweden (Folkhälsomyndigheten, 2022). Of the 68 STEC strains with complete characterisation (*stx-subtype* and *eae*) isolated from human HUS cases 2016 – 2021, 66 strains could be classified. The remaining 20 food isolates and two human isolates could not be classified, because sufficient clinical data for these genotypes were not reported in EFSA (2020)(Table S1).

Two examples are provided to illustrate the separation of STEC genotypes into two or four classes selecting arbitrary limits of the public health burden. The two-class limits were defined using a cost-benefit analysis based on the proportion of food samples that were classified as unsatisfactory (cost), and the proportion of strains causing severe illness (HUS) that are captured (benefit) as a function of the limit of the potential public health burden. The four class limits were defined arbitrarily grouping genotypes in the ranked list (Table 3), and were used to characterise the distribution of strains from the different foods in different PPHB classes.

3. Results and discussion

3.1. JEMRA approach in comparison with EFSA data

To evaluate the accordance between the approach of JEMRA and clinical data in EFSA (EFSA, 2020), STEC strains within classes were compared to their estimated probabilities to cause severe illness. Correspondence between a high risk class and a high probability for severe illness would indicate a risk based ranking. In the comparison, STEC genotypes were classified based on the gene resulting in the class associated with the greatest risk. Thus, a STEC carrying *stx2a, stx2c* and *eae* is classified based on the presence of *stx2a*, not *stx2c*, and will end up in risk class 1 instead of 3 (WHO/FAO, 2019). This assumption was made since not all genotypes were represented in the JEMRA approach, and will introduce some uncertainty since gene expression may depend on the presence of other genes. The assumption is not contradicted by the clinical data in EFSA (2020), where *stx2a and eae*, alone or in combination with any other *stx* gene were associated with 46% of cases and 81% of known HUS cases. In contrast, the presence of both *stx1* and *stx2* has been reported to reduce the proportion of infected cases developing HUS (Ardissino et al., 2020), and the presence of *stx1a* reduced the patogenicity of *stx2a* in mice (Petro et al., 2019).

Except for the high risk level 1 in the JEMRA proposal other levels (levels 2 to 5) do not correspond to a consistently decreasing potential to cause severe illness (Table 1). For instance, the probability of *stx2a* carrying STEC strains to cause HUS, hospitalisation or BD in level 5 is about the same as for *stx2d* carrying strains in level 2 (Table 1).

3.2. New approach to classify STEC strains based on potential public health burden

3.2.1. Data

Data on the proportion of human cases with the clinical outcomes of HUS, BD, and hospitalisation for different infecting STEC genotypes were used as reported in EFSA (2020). Information on the public health burden associated with the clinical outcomes, expressed in terms of

Table 2
Public health burden (DALY per case with outcome) associated with different clinical outcomes following infection with STEC.

Clinical outcome	DALY per case	Source
Non-bloody diarrhoea	0.0006	(Havelaar et al., 2004)[1]
Bloody diarrhoea	0.013	(Havelaar et al., 2004)[1]
hospitalisation	0.0048	(Mangen et al., 2017)[2]
haemolytic uraemic syndrome, hus	2.7	(Havelaar et al., 2004)[1]

[1] Estimated from Table 6 in that study by dividing the average DALY by the number of estimated cases.
[2] Estimated by multiplying severity factor 0.238 by duration 0.02 years.

DALY, was extracted from the literature. For the purpose of illustrating the method this approach was considered sufficient. Since the relative magnitudes between the clinical outcomes are more important for ranking purposes than the absolute magnitudes, DALY estimates were extracted from two studies that used similar assumptions and methodology and were from the same country, the Netherlands (Table 2). The estimated DALY per case of HUS is around 200 times that of bloody diarrhoea, and 560 times that of hospitalisation. There may be some overlap between the clinical outcomes, especially between hospitalisation and the other outcomes, and consequently, more elaborate estimates can improve the comparisons.

3.2.2. *Ranking*

STEC genotypes for which there was information in EFSA (2020), in total 18, were ordered in descending order based on the largest potential public health burden of any of the clinical outcomes (Table 3).

3.2.3. *Classes*

Any limits can be defined based on risk management considerations.

Table 3
Ranking of STEC genotypes based on potential public health burden per case. Potential public health burden is estimated as the proportion of infected cases affected by the outcome multiplied by the associated public health burden in terms of DALY for HUS, hospitalisation or bloody diarrhoea (EFSA, 2020). The STEC genotypes are arranged in descending potential public health burden expressed as mDALY (10^{-3} DALY) per case. The last column indicate which outcome was associated with the largest public health burden and thus, the outcome determining the ranking order. Several genotypes cannot be ranked because of missing data (Table S1). ND=No data.

STEC genotype		Proportion of cases with outcome			Potential public health burden (mDALY per case)			Outcome with largest public health burden
Shiga toxin (*stx*)	*eae*	HUS	HOSP	BD	HUS	HOSP	BD	
stx2a + *stx2c*	*eae*	0.290	0.571	0.655	784	2.7	8.5	HUS
stx2a	*eae*	0.274	0.564	0.584	739	2.7	7.6	HUS
stx1a + *stx2a*	*eae*	0.208	0.593	0.566	560	2.8	7.4	HUS
stx2a	–	0.104	0.320	0.263	281	1.5	3.4	HUS
stx2d	–	0.103	0.333	0.160	279	1.6	2.1	HUS
stx2c	–	0.050	ND	ND	135	ND	ND	HUS
stx1a + stx2a	–	0.045	ND	ND	123	ND	ND	HUS
stx2c	*eae*	0.043	0.198	0.239	117	0.9	3.1	HUS
stx2f	*eae*	0.038	0.210	0.087	104	1.0	1.1	HUS
stx1a	*eae*	0.012	0.276	0.273	32	1.3	3.6	HUS
stx1c + *stx2b*	–	0.010	0.146	0.181	27	0.7	2.4	HUS
stx1a + *stx2c*	*eae*	0.008	0.319	0.675	22	1.5	8.8	HUS
stx1c	–	0.006	0.189	0.195	17	0.9	2.5	HUS
stx2b	–	0.005	0.213	0.105	13	1.0	1.4	HUS
stx2e	–	0.000	ND	0.318	0	ND	4.1	BD
stx1a + *stx2b*	–	0.000	0.064	0.167	0	0.3	2.2	BD
stx2g	–	0.000	ND	0.100	0	ND	1.3	BD
stx1a	–	0.000	0.207	0.080	0	1.0	1.0	BD

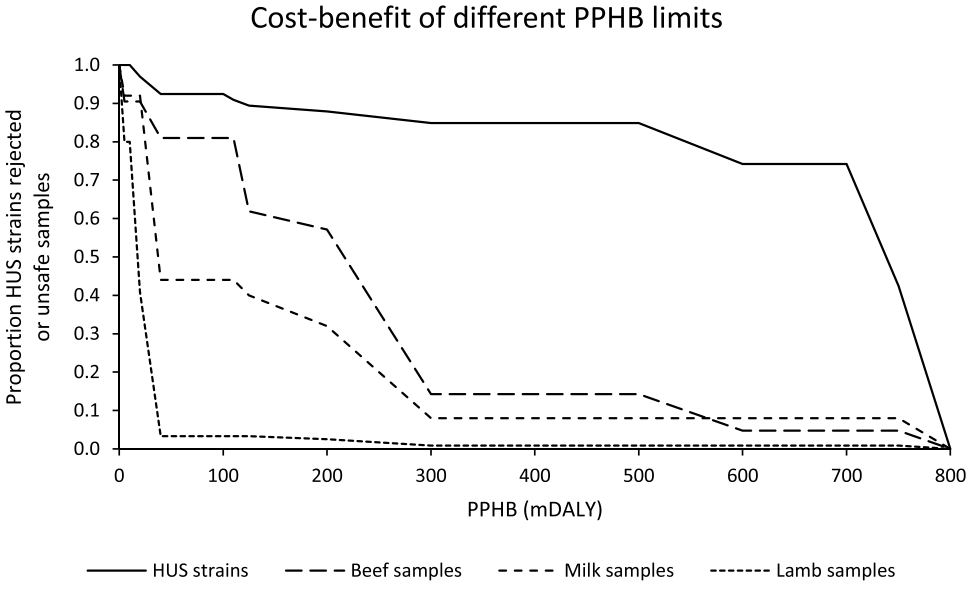

Fig. 1. A cost-benefit analysis, using the new approach for ranking of STEC genotypes, as the basis for classifying STEC positive food samples as satisfactory or unsatisfactory. The curves shows the relation between different limits between acceptable and unacceptable potential public health burden (x-axis) on the cost (proportion of samples being considered unsatisfactory) and benefit (the proportion of STEC strains associated with HUS cases in Sweden being rejected). The curves reflects the distribution of potential public health burden of the food and human strains evaluated in this study.

Table 4
Classification of STEC genotypes based on potential public health burden per case. The limits were selected arbitrarily for illustrative purposes.

Risk class	Potential public health burden (mDALY per case)[1]	STEC genotype
1	≥ 400	*stx1a* + *stx2a* + *eae*; *stx2a* + *stx2c* + *eae*; *stx2a* + *eae*
2	≥ 200, < 400	*stx2a*; *stx2d*
3	≥ 20, < 200	*stx2c* ± *eae*; *stx1a* + *stx2a*; *stx2f* + *eae*; *stx1a* + *eae*; *stx1c* + *stx2b*; *stx1a* + *stx2c* + *eae*
4	< 20	*stx1c*; *stx2b*; *stx2e*; *stx1a* + *stx2b*; *stx2g*; *stx1a*

[1] mDALY (milliDALY) = 10^{-3} DALY, disability adjusted life years.

3.3. Evaluation of new approach

STEC strains isolated from food or human HUS cases, characterised in terms of the *stx*- and *eae*-genes present, and for which clinical data were reported in EFSA (2020) were used to illustrate the new approach. The clinical data reflect surveillance and health systems in the countries reporting TeSSY data and as such can be associated with limitations in terms of *e.g.*, bias and underreporting, which was discussed in EFSA (2020). For instance, there was full genotype information for 3942 of a total of 29 945 cases, and information about clinical outcomes for between 57% (hospitalisation) and 80% (HUS) of cases. Thus, the outcome with the highest coverage was determining the ranking order of most genotypes but bias in reporting of outcomes may impact ranking order (Table 3). However, this is the best available data and the results of genotypes for which data were available were interpreted as representing the best current estimate of the probability that a human STEC case infected with the given *stx/eae* genotype shows a given clinical outcome. However, people who are exposed to STEC but not infected, and infected persons that do not end up as confirmed cases, are not included in the TESSy database (EFSA, 2020). The approach is flexible and if better data becomes available or if national data is preferred, the ranking can be updated.

From a risk management perspective, setting the limit between a satisfactory or unsatisfactory result of an analysis of a food sample, is a trade-off between the potential health impact and the cost of declaring the result unsatisfactory. For instance, under the assumption that each strain represent one food sample, an approach for setting limits between STEC classes is shown in Fig. 1. Here the context is to assess an analytical sample and the food it represents as satisfactory or unsatisfactory, *i.e.* a two-class system. The cost when deciding on the limits is the proportion of food samples declared as unsatisfactory, *i.e.*, from which STEC strains being classified as unsatisfactory in terms of the potential public health burden associated with an infection, have been isolated. The cost need to be balanced with the benefit, *i.e.* the proportion of HUS causing strains, which would be captured in the samples classified as unsatisfactory. In Fig. 1, the cost depending on the limit between unsatisfactory and satisfactory in terms of potential public health burden is shown per food type and the benefit in terms of proportion of HUS cases that would be captured is shown. A limit of 500 mDALY, targeting genotypes *stx2a*-+*eae*+other *stx* genes (Table 3), would lead to 14% or less of these food samples being considered unsatisfactory (cost) and about 85% of STEC strains having caused HUS in Sweden would be captured (Fig. 1). Limits lower than 200 mDALY, targeting also *eae*-negative *stx2a* and *stx2d* (Table 3), would capture 88% of HUS strains and declare 57% of beef samples as unsatisfactory. This type of graph can be used to transparently discuss the basis for risk management decisions.

As another example, STEC genotypes were separated into four classes (Table 4), based on an arbitrary grouping of estimated potential public health burdens shown in Table 3. A graph of the distribution of STEC isolates in classes from different food sources based on the arbitrary class limits is shown in Fig. 2. The figure provides a profile of the distribution of strains in terms of potential public health burden. Amongst the food types, the proportion of strains with a high potential (class 1) to cause severe illness was lowest for lamb meat, and highest for beef and unpasteurised milk. A comparison with the distribution of STEC strains from HUS cases provides a basis for evaluating the selected limits for the

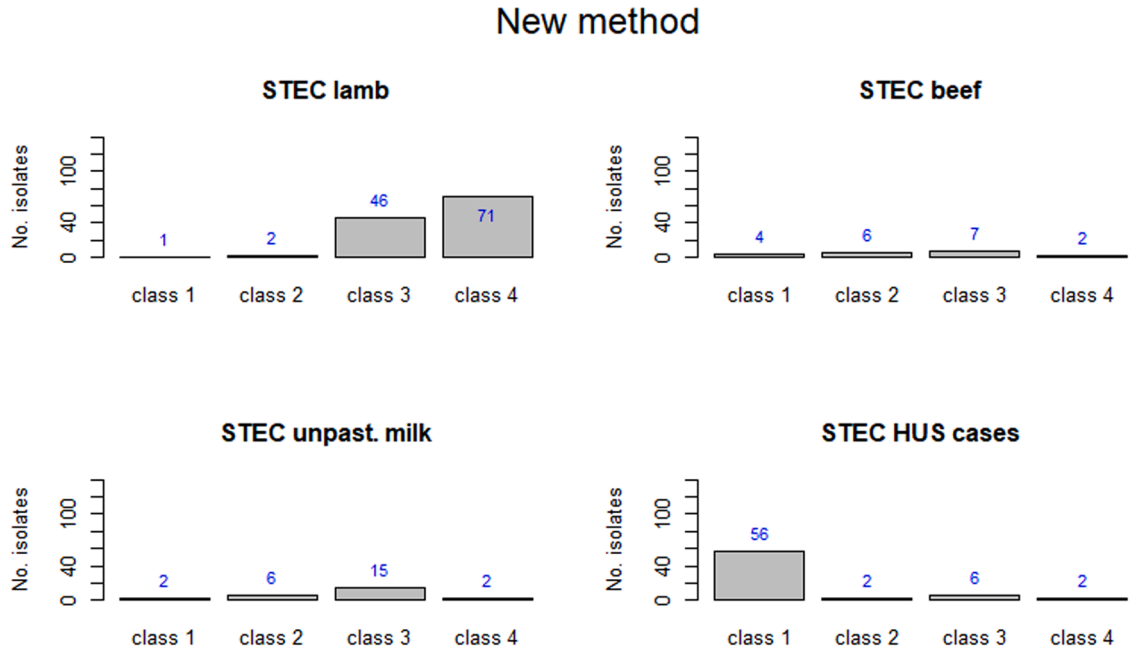

Fig. 2. Distribution of STEC genotypes isolated from samples of lamb meat, beef, unpasteurised milk, and from cases with HUS, in different classes according to a new approach based on the estimated potential public health burden per case. Arbitrary class limits are shown in Table 4.

risk classes in terms of the ability of the system to classify HUS causing STEC strains. A majority of the human isolates were classified in the highest risk class (Fig. 2).

4. Conclusion

Existing approaches do not unequivocally classify different STEC genotypes according to their probability to cause severe illness. The new approach addresses that limitation, improves transparency of risk management decisions and is risk based in terms of the probability and consequences following infection (severe illness). It is not risk based in terms of the risk associated with the presence of a STEC genotype in the food since this would involve genotype specific risk assessments considering exposure which would involve more work and data that are not always available.

CRediT authorship contribution statement

Roland Lindqvist: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Catarina Flink:** Methodology, Writing – original draft, Writing – review & editing. **Mats Lindblad:** Methodology, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Authors have no conflicts of interest.

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mran.2023.100246.

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