Journal of Dairy Research

cambridge.org/dar

Disinfection reduces but does not eliminate drug resistant *Escherichia coli* from livestock trailers following transport of calves

Joanna Wioletta Urbaniec, Peers L. Davies and Joseph M. Neary

Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK

Research Article

Cite this article: Urbaniec JW, Davies PL and Neary JM (2025) Disinfection reduces but does not eliminate drug resistant *Escherichia coli* from livestock trailers following transport of calves. *Journal of Dairy Research*, 1–6. https://doi.org/10.1017/S0022029925101581

Received: 28 April 2025
Revised: 18 July 2025
Accepted: 12 September 2025

Keywords:
AMR; biosecurity; food supply chain; livestock transport; multidrug resistance

Corresponding author:
Joanna Wioletta Urbaniec;
Email: asia.urbaniec@gmail.com

Abstract

This research article addresses the hypothesis that vehicles used for cattle transport are contaminated with *Escherichia coli*, a potential foodborne pathogen, despite current regulations on sanitation practices. Dairy cattle and calves are regularly transported to auction markets, calf rearers and slaughterhouses. UK Government guidelines require livestock transport vehicles to be cleaned and disinfected within 24 hours of use or before re-use within that period. It is feasible, however, that if cleaning fails to eradicate bacteria, then transport vehicles can act as a fomite in the spread of antimicrobial resistance (AMR) pathogens. In this study, 13 trailer-loads (TLs) of calves were transported for 40–60 minutes. Trailers were then cleaned and disinfected within 20 minutes of unloading. Five sites within the trailer were swabbed after pressure washing and again 30 minutes after application of disinfectant. A bacterial count for *E. coli* was performed through growth on selective agar, and species identification was confirmed by MALDI-TOF. A subset of 30 isolates was selected for antibiotic susceptibility screening to a panel of veterinary and human antibiotics. *E. coli* were recovered from all TLs and sites; however, not all sites were contaminated in each TL. *E. coli* count was significantly reduced, but not eliminated, following application of disinfectant. Furthermore, high prevalence of resistance to sulphonamides, first-generation cephalosporins, and tetracyclines was observed. Forty percent of screened isolates were also classified as multidrug-resistant (MDR) (i.e. resistant to at least one antibiotic from three or more antibiotic classes). Application of disinfectant did not increase the risk of recovering an MDR isolate. This study demonstrates that livestock trailers can harbour potential zoonotic pathogens with AMR properties. Disinfection in accordance with current guidelines is an important step in reducing, but not eradicating, bacterial populations in these vehicles. Improved cleaning and/or disinfection policies are required to mitigate the potential for AMR transmission.

Introduction

In 2020, there were 11,900 registered dairy holdings in the United Kingdom (Uberoi, 2021). Cattle and calves are regularly transported from these holdings to other farms, auction markets and slaughterhouses, equating to thousands of trips each month. Animals can be transported by private vehicles but often, especially for larger holdings, they are picked up by professional livestock hauliers who visit multiple holdings across a wide geographical area. This raises concern on whether animal transport can contribute to the spread of zoonotic pathogens and AMR between holdings, and on to the food production chain. Recently, the European Food Safety Authority Panel on Biological Hazards released an opinion article which concluded that livestock transport is 'almost certain' to contribute to the spread of antibiotic resistance (BIOHAZ, 2022). Furthermore, the report highlights the 'urgent need' for a direct assessment of the impact of livestock transport on AMR spread, as there is a lack of direct observational studies in this field. Previous work conducted on pigs (Abdalla *et al.*, 2021; Galvis and Machado, 2024) and poultry (Rule *et al.*, 2008) found transport vehicles to be a source of pathogenic and/or antibiotic-resistant microorganisms; however, evaluation of risk during cattle transport, especially in the United Kingdom, is lacking. Studies of American veal production have reported calf transport trailers to harbour AMR *E. coli* and *Salmonella spp.* (Locke *et al.*, 2022; Dunmyre, 2024), although the efficacy of cleaning regimes of those trailers was not investigated.

The UK government guidelines stipulate that any vehicle used to transport livestock needs to be cleaned within 24 hours of use, or prior to re-use, whichever comes first. Cleaning of the interior with water 'until it is free of dirt' must always be completed, and disinfectant should be applied 'where appropriate', but is not required (The Transport of Animals (Cleansing and Disinfection) Order, 2000). A recent survey conducted in abattoirs suggests low compliance

Table 1. Calf transports investigated as a part of this study. A: 2.4 × 1.5 m, B: 3.7 × 1.8 m and C: 3.0 × 1.5 m twin-axle trailer. Samples from the same study group were collected within 5 days of each other

Load	Farm	Journey duration (min)	Number of calves	Trailer	Study group
1	A	30	12	A	1
2	D	20	7	A	1
3	H	50	7	A	1
4	F	20	4	A	1
5	D	20	7	A	2
6	E	45	7	A	2
7	F	20	4	A	2
8	A	30	12	B	2
9	G	90	10	B	2
10	A	30	12	C	3
11	B	90	10	C	3
12	C	160	7	A	3
13	D	20	7	A	3

amongst hauliers (Weber and Meemken, 2018); furthermore, even if cleaning and disinfection guidelines are closely followed, it is likely that not all potential pathogens will be removed.

The aim of this study was to evaluate the level of microbiological contamination of trailers used to transport cattle, following cleaning and disinfection as per current UK government guidelines. We focus on contamination with *Escherichia coli* as a sentinel species, as it is a potential foodborne pathogen known to easily acquire antibiotic resistance genes (Poirel *et al.*, 2018). FAM30® was selected as the disinfectant of choice as it is commonly used by livestock farmers and veterinarians and is approved by the Department of Environment, Food & Rural Affairs for general disinfection purposes (DEFRA, 2025). We hypothesised that even if government guidelines are strictly followed, cleaning and disinfection would reduce but not eradicate *E. coli* from trailer surfaces, with concealed areas showing less of a reduction in *E. coli* counts post-disinfection than exposed areas.

Materials and methods

Animal transport, trailer cleaning and sampling

A convenience sample of trailer journeys associated with another study (grant no. BB/Y006887/1) was investigated. Thirteen trailerloads (TLs) of weaned 10–12 weeks old Angus x Holstein calves were transported by the authors from dairy farms (n = 8) to a livestock research facility at the University of Liverpool (UoL), Leahurst Campus in Neston, Cheshire (Table 1). Farms were located in North Wales (Farm D), Cheshire (Farms A/E/F/H), Staffordshire (Farm B), Yorkshire (Farm C) and Shropshire (Farm G). Trailers A and B were owned by UoL (farm animal practice trailer and university-operated mixed livestock farm trailer, respectively). Trailer C was owned by one of the authors (JN) who owns a beef cattle farm in North Wales. Trailers were cleaned prior to being used as described below. There was at least 3 hours of downtime between the end of cleaning and the collection of the subsequent TL of calves.

Trailers were washed by an experienced livestock technician within 20 minutes of calf unloading at the research facility, at a designated location situated on a concrete floor sloping towards a central drain. First, the trailer was rinsed using either a pressure washer or high-flow hose to flush most of the faecal material from the trailer. Mains tap water was used throughout the cleaning process. A washing brush was then used to scrub areas of faecal material that were adhered to the inside of the trailer. Next, the trailer was sprayed with a pressure washer (Karcher HDS 5/12 C) at 60°C starting at the back wall and working towards the trailer ramp. Within 5 minutes of the final pressure wash, five sites within the trailer were swabbed three times whilst rotating the tip (10 cm x 5 cm area) with an autoclaved rayon-tipped swab (McKesson Medical) pre-dipped in sterile phosphate buffered saline (PBS): top of the outside railing, ramp, inside railing, back wall and underneath side panel (Supplementary Figure 1). Sampling sites were pre-marked with a permanent marker pen to ensure a consistent size of the swabbing area. The inside of the trailer was then sprayed with a commonly used multi-purpose iodophor disinfectant (FAM® 30, Evans Vanodine International Plc., Preston, UK). A 1:100 disinfectant to water dilution and 30-minute contact time was used as per the manufacturer's guidelines. The disinfectant was sprayed onto the trailer surfaces using a 10 L hand-pumped pressure washer with a full cone spray nozzle in the same sequence as pressure-washing (back wall to front ramp) and left to dry for 30 minutes. Afterwards, swabbing was repeated on corresponding sites (e.g. if the left side railing was swabbed prior to disinfection, the right side railing was swabbed after), as it was reasoned that swabbing the same site twice would result in a reduced bacterial count due to the physical removal of bacteria by the initial swab, regardless of the action of the disinfectant.

Swabs were stored in 3 mL of PBS at 4°C and plated on agar plates on the same day. An air swab opened inside the trailer for 1 minute was used as the negative control.

E. coli isolation and antibiotic susceptibility screening

Swabs suspended in PBS were vortexed for 1 minute, and 100 µL of PBS was plated on Harlequin *E. coli*/coliform chromogenic selective agar (HECA) (Neogen). Plates were incubated aerobically for 16–20 hours at 37°C. Colonies were counted with a colony counter, where on HECA agar purple colonies were classified as *E. coli*, blue as non-*E. coli* coliforms and white as non-coliforms, according to the agar manufacturer's instructions. A total of 30 presumed *E. coli* isolates (10 per study group) were selected for further investigation stratified by TL, site, and disinfection status (pre-/post-FAM application in the trailer). Within each study group, presumed *E. coli* colonies were selected so that one isolate from each site pre- and post-disinfection and at least one isolate from each TL was included, if possible. If no isolates were recovered from a given site at a certain disinfection status or from a given TL, a colony from a different plate was selected at random to maintain an equal number of isolates between study groups. This meant that the number of isolates was not necessarily equal between sites and TLs within the study group.

Species identification was confirmed by matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) at the UoL Veterinary Microbiology Diagnostic laboratory, and isolates were subsequently screened for their antibiotic susceptibility profiles with the Sensititre broth microdilution system (ThermoFisher Scientific): Bovine/Porcine BOPO6 Vet AST plate and Gram Negative GN3F plate, according to

https://doi.org/10.1017/S0022029925101581 Published online by Cambridge University Press

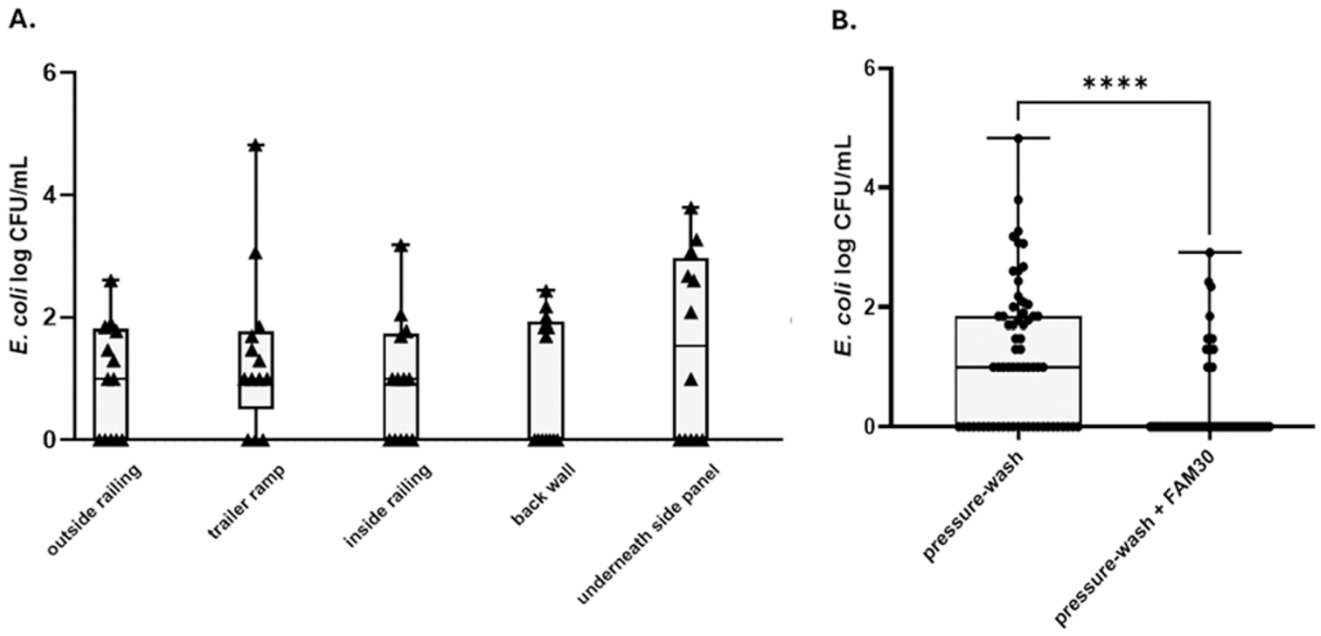

Figure 1. Boxplot of *E. coli* CFU/mL: A. recovered from different sites within the trailer and B. recovered across all sites within the trailer prior (pressure-wash) and post (pressure wash + FAM30) disinfection. Boxes represent 25th to 75th percentiles, with line at the median. Range is represented by bars. **** = $p < 0.0001$ by Wilcoxon matched-pairs signed rank test, with match-pairs assigned for each site within trailer load prior/post-disinfection.

manufacturer's instructions. Resistance breakpoints were obtained from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) v.14.0 clinical breakpoints table (EUCAST, 2024). Isolates classified as 'intermediate' were grouped separately from resistant isolates. Extended spectrum β-lactamase (ESBL) and carbapenem resistant *Enterobacteriaceae* (CREs) were classified according to EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance version 2.0 (EUCAST, 2017).

FAM30 disinfectant susceptibility was investigated using a broth microdilution method as described previously (Andrews, 2001). Briefly, bacterial cultures were grown in Mueller Hinton (MH) broth overnight, at 37°C and 200 rpm shaking. Cultures were then diluted 1:200 in fresh media and incubated for 2–3 hours until they reached mid-log phase of growth ($OD_{600} \sim 1$–1.2). Cultures were then diluted to 0.5 McFarland standard and further diluted 1:1000 in MH broth for a final inoculum size of $\sim 10^5$ CFU/mL. Within 30 minutes of preparation, the inoculum was added at 1:1 v/v to a 96-well plate containing 1:25 and 1:50 dilutions of FAM30 disinfectant in MH broth (starting at 2x the highest concentration assayed). Assayed concentrations were selected as per manufacturer's instructions for standard cleaning and disinfection (1:100) and general order disease outbreaks (1:50) (Evans Vanodine). Plates were incubated aerobically for 24 hours at 37°C. Following incubation, plates were inspected visually for the presence of turbidity. Each isolate was assayed in duplicate, with three technical replicates of each biological replicate. Minimum inhibitory concentration was the lowest concentration that inhibited the growth of both replicates.

Calf treatment history and feeding of waste milk

Antibiotic treatment records of the calves transported in our study were provided by the participating farms. Individual animal veterinary medicine records must be kept for food-producing animals in the United Kingdom for at least 5 years. The feeding of waste milk from cows treated with antibiotics was also recorded.

Statistical analysis

The amount of *E. coli* recovered among the sampled sites was compared with a Kruskal–Wallis test with Dunn's correction for multiple comparisons. Hierarchical clustering was not performed due to co-linearity between clustering variables (the largest trailer was always used to collect the greatest number of calves which originated from the same farm and *vice versa*); however, this did not violate the assumptions of the Kruskal–Wallis test. The effect of disinfection on *E. coli* count was determined using the Wilcoxon matched-pairs signed rank test, with paired values of *E. coli* CFU/mL before/after disinfection for each site within a TL. The proportion of recovered multidrug-resistant (MDR) isolates and sites with undetectable *E. coli* count before or after disinfection was compared with Fisher's exact test. All statistical analyses were performed in GraphPad Prism v.10.2.3 (GraphPad Software LLC).

Results

E. coli load did not differ amongst sites within the trailer and was reduced by application of disinfectant

E. coli were recovered from all trailer loads and across all sites before and after disinfection; although, not all sites were contaminated in each TL. Values ranged from < 10 CFU/mL to 6.3×10^3 CFU/mL, with the overall median of 10 CFU/mL (Fig. 1A). The amount of recovered *E. coli* did not differ among sampled sites ($p > 0.99$). No *E. coli* were recovered from 39% (25/64) and 78% (50/64) of sampled surfaces before and after the application of disinfectant respectively, within the limit of detection of 10 CFU/mL. Application of disinfectant reduced the overall

https://doi.org/10.1017/S0022029925101581 Published online by Cambridge University Press

median *E. coli* count by 10-fold, ($p < 0.0001$), and doubled the number of sites with an undetectable *E. coli* count ($p < 0.0001$) (Fig. 1B).

AMR E. coli were recovered from livestock trailers before and after application of disinfectant

Species identity of 30 presumed *E. coli* isolates, chosen randomly across all sites and TLs, was confirmed by MALDI-TOF spectrophotometry. One isolate subsequently failed to grow in culture and therefore 29 of the original 30 isolates were exposed to a panel of 10 veterinary-use and 23 human-use antibiotics, as well as manufacturer-recommended working concentrations of FAM30 (Supplementary Table 1). Antibiotics with the highest prevalence of resistance were sulfadimethoxine (93%) and cephalothin (55%), followed by tetracyclines (34%), gentamicin (20%), ampicillin (17%), cefazolin (14%) and trimethoprim/sulphamethoxazole (14%). No ESBLs (isolates resistant to third-generation cephalosporins: cefpodoxime, ceftazidime and ceftriaxone), or CREs (isolates resistant to meropenem and ertapenem) were recovered. All isolates failed to grow in the presence of disinfectant at assayed concentrations.

Twelve isolates, each from a different TL, were classified as multidrug resistant, that is, resistant to at least one antibiotic from three or more classes. The proportion of isolates exhibiting MDR was 45% (5/11 isolates) and 39% (7/18 isolates) post and prior to disinfection, respectively (RR: 1.17, $p > 0.99$). The proportion of MDR isolates recovered following transport from different farms varied from 100% MDR (Farms C and H), 50% MDR (Farms B, D and F), 25% MDR (Farm A), to 0% MDR (Farms E and G) (Table 2).

Antibiotic treatment history did not appear to directly influence E. coli resistance profiles

At least one calf had been treated with antibiotics prior to transport in six out of thirteen TLs (Supplementary Table 2). Antibiotics used were oxytetracycline (TLs 2 and 13), tulathromycin (TLs 7 and 9), cloxacillin (TL 8) and florfenicol (TL 11).

Tulathromycin, oxytetracycline and florfenicol were part of the antibiotic susceptibility panel in this study. Prevalence of AMR to the aforementioned antibiotics did not appear to be dependent on previous exposure. No resistance to florfenicol was observed in TL 11 isolates, compared to 14% overall resistance. Two out of three (66%) isolates from TL 2 were resistant to tetracyclines, compared to 34% overall resistance. There is no clinical resistance breakpoint available for *E. coli* for tulathromycin; however, MIC values of TLs 7 and 9 were comparable to the overall MIC values for this antibiotic (medians of 2.5 and 2, respectively).

Discussion

Livestock transport vehicles can act as fomites for pathogens and AMR

This study demonstrated that livestock trailers are likely to be contaminated with antibiotic-resistant *E. coli*, even when current disinfection guidelines are strictly followed. It is likely that in a practical setting, the levels of contamination may be greater than reported here, as compliance with sanitation guidelines will vary (Weber and Meemken, 2018). Therefore, if we assume that some degree of contamination is unavoidable, effective risk reduction measures should begin upstream in the food production process. Numerous studies have shown that increased antibiotic use on farms increases shedding of antibiotic-resistant pathogens (Avrain *et al.*, 2003; Brunton *et al.*, 2014; Cameron-Veas *et al.*, 2016), as well as increases the risk of spread of AMR genes via horizontal gene transfer (Heuer and Smalla, 2007). Reducing antibiotic use in livestock would reduce shedding of AMR pathogens during transport and subsequently reduce vehicle contamination.

Proper cleaning practices are nonetheless important, and here we demonstrated that use of iodophor disinfectant, applied in the form of a full cone spray, significantly reduced *E. coli* count within the trailer. Currently, the use of chemical disinfectants for routine cleaning of livestock transport vehicles is not mandatory in the United Kingdom (The Transport of Animals Order, 2000), and can raise concerns regarding environmental contamination, increased costs and increased risk of resistance development. Iodophors, including FAM30, are known to corrode and stain equipment, and can cause serious skin and eye irritation if not handled properly; consequently, staff training and use of appropriate personal protective equipment should be included in farm risk management considerations. Another potential issue is the potential for co-selection of AMR with disinfectant resistance genes encoded on MDR plasmids (Sheikh *et al.*, 2025); however, we did not recover a higher proportion of MDR isolates following disinfectant application, and all screened *E. coli* isolates failed to grow in the presence of disinfectant used at manufacturer-recommended concentrations. Our results suggest that chemical disinfection following pressure-washing is beneficial; however, larger-scale investigations, including evaluation of different application methods and disinfectant types, are needed to validate these conclusions.

Escherichia coli isolated from livestock transport vehicles harbours resistance to human and veterinary antibiotics

Although *E. coli* is intrinsically susceptible to nearly all commonly used antibiotics, MDR and extensively-drug resistant isolates are recovered at increasing frequency which poses significant public health concerns (Wang *et al.*, 2020; Simner *et al.*, 2023). Whilst antibiotics licenced in human medicine are not commonly licenced for veterinary use, resistance mechanisms to both groups can overlap, especially if it is mediated via MDR plasmids (Poirel *et al.*, 2018). In this study, we demonstrated that *E. coli* recovered from livestock trailers can carry resistance to multiple human and veterinary antibiotics. Almost all isolates were resistant to sulfadimethoxine (brand name Albon), a sulphonamide licensed in the United Kingdom to treat respiratory infections, calf diphtheria, foot rot and less commonly coccidiosis (Zoetis, 2022). Conversely, we observed moderate resistance to sulfamethoxazole/trimethoprim (Bactrim), a sulphonamide commonly prescribed in human medicine for urinary tract infections, most often caused by *E. coli* (Flores-Mireles *et al.*, 2015). Although sulfamethoxazole is not approved for veterinary use, cross resistance between different sulphonamide antibiotics is common (Skold, 2001). Approximately half of the isolates were resistant to cephalothin, a first-generation cephalosporin that is most used for surgery prophylaxis and treatment of a broad range of infections in human and veterinary medicine (Del Pilar Zarazaga *et al.*, 2024). However, apart from one isolate, we did not observe resistance to second-/third-generation cephalosporins. Interestingly, we did not observe high resistance levels to ampicillin, suggesting a cephalothin resistance mechanism other than β-lactamase production, as both antibiotics possess a β-lactam ring as part of their structure (Cho and Kim, 2018). Finally, we observed a moderate prevalence of resistance

https://doi.org/10.1017/S0022029925101581 Published online by Cambridge University Press

Table 2. Prevalence of antibiotic resistance amongst *E. coli* recovered from calf transport trailers. Prevalence expressed as ratio of resistant isolates to the total number of isolates recovered from transport(s) from given farm. Veterinary use antibiotics are underlined. Clinical resistance breakpoints were obtained from EUCAST v.14.0 (European Committee on Antimicrobial Susceptibility Testing, 2024); for danofloxacin, norfloxacin and spectinomycin epidemiological cut-off values were used due to lack of established clinical breakpoints (Tomazi *et al.*, 2018); for tulathromycin MIC range was provided due to lack of either

	Farm A	Farm B	Farm C	Farm D	Farm E	Farm F	Farm G	Farm H
Ceftiofur	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Chlortetracycline	**2/8**	**1/4**	0/2	**2/4**	**1/3**	**3/4**	0/2	**1/2**
Danofloxacin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Enrofloxacin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Florfenicol	0/8	0/4	0/2	0/4	0/3	0/4	0/2	**1/2**
Neomycin	0/8	0/4	0/2	**1/4**	0/3	0/4	0/2	0/2
Oxytetracycline	**2/8**	**1/4**	0/2	**2/4**	**1/3**	**3/4**	0/2	**1/2**
Spectinomycin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Sulphadimethoxine	**7/8**	**4/4**	**2/2**	**4/4**	**2/3**	**4/4**	0/2	**2/2**
Tulathromycin*	(≤1- 16)	(2–4)	(4)	(≤1 – 2)	(≤1 – 2)	(≤1 – 2	(2–4)	(≤1 – 2)
Amikacin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Ampicillin	**1/8**	0/4	**1/2**	0/4	0/3	0/4	0/2	**1/2**
Ampicillin/Sulbactam	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Aztreonam	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Cefazolin	**1/8**	0/4	0/2	**1/4**	0/3	0/4	0/2	**1/2**
Cefepime	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Cefoxitin	**3/8**	**2/4**	**1/2**	0/4	0/3	0/4	0/2	0/2
Cefpodoxime	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Ceftazidime	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Ceftriaxone	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Cefuroxime (oral)	0/8	0/4	**2/2**	**1/4**	0/3	0/4	0/2	0/2
Cefuroxime (parenteral)	0/8	0/4	**2/2**	**1/4**	0/3	0/4	0/2	0/2
Cephalothin	0/8	**2/4**	**1/2**	**2/4**	**1/3**	**3/4**	**2/2**	**1/2**
Ciprofloxacin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Ertapenem	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Gentamicin	**1/8**	**2/4**	**1/2**	**1/4**	0/3	0/4	0/2	0/2
Meropenem	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Piperacillin/tazobactam	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Tetracycline	**2/8**	**1/4**	0/2	**2/4**	**1/3**	**4/4**	0/2	0/2
Ticarcillin/clavulanic acid	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Tigecycline	0/8	0/4	**1/2**	**1/4**	0/3	0/4	0/2	0/2
Tobramycin	0/8	0/4	0/2	0/4	0/3	0/4	0/2	0/2
Trimethoprim/sulfamethoxazole	**1/8**	**1/4**	0/2	0/4	0/3	0/4	0/2	**1/2**

to tetracyclines and gentamycin. Tetracyclines are no longer recommended to treat *E. coli* infections in humans due to a high prevalence of resistance; however, they are still commonly prescribed in veterinary medicine and are considered a first-line antibiotic choice (Category D) under the European Medicines Agency, likely maintaining positive selective pressure on resistant isolates (Karami *et al.*, 2006; Kimura *et al.*, 2022). Reassuringly, we did not isolate any ESBLs or CREs from the trailers in our study. However, an MDR isolate was recovered following a trip to six out of eight farms, irrespective of the antibiotic treatment history of transported animals. Notably, one isolate exhibited resistance to 14 out of 29 tested antibiotics. Although the animals participating in this study had not been treated with these antibiotics, the farm environment can facilitate the dissemination of MDR plasmids via surfaces (e.g. equipment, clothing or bedding), feed (e.g. waste milk) or waste (e.g. wastewater and manure). Therefore, strict biosecurity practices, including effective cleaning of transport vehicles, together with antibiotic stewardship, are crucial to minimise the risk of AMR spread onto the food production chain.

https://doi.org/10.1017/S0022029925101581 Published online by Cambridge University Press

Altogether, our study demonstrates that *E. coli* found in 'clean' livestock transport trailers harbour resistance to multiple clinically relevant antibiotics. Isolates recovered from those vehicles are a potential health hazard not only to stockpersons but also to the public, as they have the potential to spread during animal transport through persistent low-level contamination.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029925101581.

Acknowledgements. This work was supported by Biotechnology and Biological Sciences Research Council (award number: BB/Y006887/1), and University of Liverpool Faculty of Health and Life Sciences Research and Impact Directorate Early Career Fund. Authors would like to gratefully acknowledge Giorgia Cavalli and University of Liverpool Veterinary Microbiology Diagnostics Laboratory for their technical assistance on this project.

Ethical statement. Animal transport was conducted in association with Home Office Project Licence PP6326528.

References

Abdalla SE, Abia ALK, Amoako DG, Perrett K, Bester LA and Essack SY (2021) From farm-to-fork: *E. coli* from an intensive pig production system in South Africa shows high resistance to critically important antibiotics for human and animal use. *Antibiotics* **10**(2), 178. https://doi.org/10.3390/antibiotics10020178

Andrews JM (2001) Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*. 48(suppl_1), pp. 5–16. Available at https://doi.org/10.1093/jac/48.suppl_1.5.

Avrain L, Humbert F, L'Hospitalier R, SandersP, Vernozy-Rozand C and Kempf I (2003) Antimicrobial resistance in *Campylobacter* from broilers: association with production type and antimicrobial use. *Veterinary Microbiology* **96**(3), 267–276. https://doi.org/10.1016/j.vetmic.2003.07.001

Brunton LA, Reeves HE, Snow LC and Jones JR (2014) A longitudinal field trial assessing the impact of feeding waste milk containing antibiotic residues on the prevalence of ESBL-producing Escherichia coli in calves. *Preventive Veterinary Medicine* **117**(2), 403–412. https://doi.org/10.1016/j.prevetmed.2014.08.005

Cameron-Veas K, Moreno MA, Fraile L and Migura-Garcia L (2016) Shedding of cephalosporin resistant *Escherichia coli* in pigs from conventional farms after early treatment with antimicrobials. *The Veterinary Journal* **211**, 21–25. https://doi.org/10.1016/j.tvjl.2016.02.017

Cho H and Kim K (2018) *Escherichia coli* OxyS RNA triggers cephalothin resistance by modulating the expression of CRP-associated genes. *Biochemical and Biophysical Research Communications* **506**(1), 66–72. https://doi.org/10.1016/j.bbrc.2018.10.084

Del Pilar Zarazaga M, Tinti MG, Litterio NJ, Himelfarb MA, San Andrés-Larrea MI, Rubio-Langre S, Serrano-Rodríguez JM and Lorenzutti AM (2024) Dose regimen optimization of cephalothin for surgical prophylaxis against Staphylococcus aureus and coagulase negative staphylococci in dogs by pharmacokinetic/pharmacodynamic modeling. *Research in Veterinary Science* **171**, 105202. https://doi.org/10.1016/j.rvsc.2024.105202

Department of Environmental, Food and Agricultural Affairs (DEFRA) (2025) *Disinfectants Approved for Use in England, Scotland and Wales*. Available at https://disinfectants.defra.gov.uk (accessed 7 2025).

Dunmyre A (2024) Characterisation of antimicrobial resistance in special-fed veal production environments. *Zoonoses and Public Health* **72**(1), 75–83. https://doi.org/10.1111/zph.13186

EFSA Panel on Biological Hazards (BIOHAZ) (2022) Transmission of antimicrobial resistance (AMR) during animal transport. *EFSA Journal* **20**(10), https://doi.org/10.2903/j.efsa.2022.7586

European Committee on Antimicrobial Susceptibility Testing (2017) *EUCAST Guidelines for Detection of Resistance Mechanisms and Specific Resistances of Clinical And/or Epidemiological Importance*. Available at https://www.eucast.org/resistance_mechanisms (accessed 2 2025).

European Committee on Antimicrobial Susceptibility Testing (2024) *Breakpoint Tables for Interpretation of MICs and Zone Diameters Version 14.0*. Available at https://www.eucast.org/clinical_breakpoints (accessed 2 2025).

Flores-Mireles AL, Walker JN, Caparon M and Hultgren SJ (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology* **13**(5), 269–284. https://doi.org/10.1038/nrmicro3432

Galvis JA and Machado G (2024) The role of vehicle movement in swine disease dissemination: Novel method accounting for pathogen stability and vehicle cleaning effectiveness uncertainties. *Preventive Veterinary Medicine* **226**, 106168. https://doi.org/10.1016/j.prevetmed.2024.106168

Heuer H and Smalla K (2007) Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months. *Environmental Microbiology* **9**(3), 657–666. https://doi.org/10.1111/j.1462-2920.2006.01185.x

Karami N, Nowrouzian F, Adlerberth I and Wold AE (2006) Tetracycline resistance in *Escherichia coli* and Persistence in the infantile colonic microbiota. *Antimicrobial Agents and Chemotherapy* **50**(1), 156–161. https://doi.org/10.1128/AAC.50.1.156-161.2006

Kimura J, Kudo H, Fukuda A, Yamada M, Makita K, Oka K, Takahashi M, TamuraY and Usui M (2022) Decreasing the abundance of tetracycline-resistant *Escherichia coli* in pig faeces during nursery using flavophospholipol as a pig feed additive. *Veterinary and Animal Science* **15**, 100236. https://doi.org/10.1016/j.vas.2022.100236

Locke SR, Pempek JA, Meyer R, Portillo-Gonzalez R, Sockett D, Aulik N and Habing G (2022) Prevalence and sources of salmonella lymph node infection in special-fed veal calves. *Journal of Food Protection* **85**(6), 906–917. https://doi.org/10.4315/jfp-21-410

Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P and Schwarz S (2018) Antimicrobial Resistance in *Escherichia coli*. *Microbiology Spectrum* **6**(4), 6.4.14. https://doi.org/10.1128/microbiolspec.ARBA-0026-2017

Rule AM, Evans SL and Silbergeld EK (2008) Food animal transport: a potential source of community exposures to health hazards from industrial farming (CAFOs). *Journal of Infection and Public Health* **1**(1), 33–39. https://doi.org/10.1016/j.jiph.2008.08.001

Sheikh M, Gholipour S, Ghodsi S and Nikaeen M (2025) Co-selection of antibiotic and disinfectant resistance in environmental bacteria: health implications and mitigation strategies. *Environmental Research* **267**, 120708. https://doi.org/10.1016/j.envres.2024.120708

Simner PJ, Bergman Y, Conzemius R, Jacobs E, Tekle T, Beisken S and Tamma PD (2023) An NDM-producing *Escherichia coli* clinical isolate exhibiting resistance to cefiderocol and the combination of ceftazidime-avibactam and aztreonam: another step toward pan-β-lactam resistance. *Open Forum Infectious Diseases.* **10**(7), 276. https://doi.org/10.1093/ofid/ofad276

Skold O (2001) Resistance to trimethoprim and sulfonamides. *Veterinary Research* **32**(3/4), 261–273. https://doi.org/10.1051/vetres:2001123

Tomazi T, Coura FM, Gonçalves JL, Heinemann MB and SantosMVD (2018) Antimicrobial susceptibility patterns of *Escherichia coli* phylogenetic groups isolated from bovine clinical mastitis. *Journal of Dairy Science* **101**(10), 9406–9418. https://doi.org/10.3168/jds.2018-14485

The Transport of Animals (Cleansing and Disinfection) (England) (No. 2) Order 2000, SI: 2000/1618.

Uberoi E (2021) *UK dairy industry statistics: Commons Library Research Briefing (No. 2721). 9 September. House of Commons Library*. Available at https://researchbriefings.files.parliament.uk/documents/SN02721/SN02721.pdf (accessed 29 September 2025).

Wang M, Wang W, Niu Y, Liu T, Li L, Zhang M, Li Z, Su W, Liu F, Zhang X and Xu H (2020) A Clinical Extensively-Drug Resistant (XDR) Escherichia coli and Role of Its β-Lactamase Genes. *Frontiers in Microbiology* **11**, 590357. https://doi.org/10.3389/fmicb.2020.590357

Weber L and Meemken D (2018) Hygienic measures during animal transport to abattoirs - a status quo analysis of the current cleaning and disinfection of animal transporters in Germany. *Porcine Health Management* **4**(1), 1. https://doi.org/10.1186/s40813-017-0078-x

Zoetis (2022) *Albon (Bolus) Information Leaflet*. Available at https://www.zoetisus.com/products/cattle/albon-boluses (accessed 2 2025).