



## Article

# Antimicrobial Resistance in Petting Zoo Animals in the United Kingdom

Alice Nishigaki , Kurt Arden and Siân-Marie Frosini \*

Department of Pathobiology and Population Sciences, Royal Veterinary College, Hatfield AL9 7TA, UK; karden@rvc.ac.uk (K.A.)

\* Correspondence: sfrosini@rvc.ac.uk

## Abstract

The role of petting zoo animals in the dissemination of disease has been widely studied, yet understanding the potential reservoir of antimicrobial resistance (AMR) in these centres has not been explored in the United Kingdom (UK). To understand the carriage of AMR pathogens within petting zoos, this study aimed to identify AMR in *E. coli* and *Staphylococcus intermedius* group (SIG) isolated from faeces and skin, respectively, including selective cultures for ESBL-*E. coli* and methicillin-resistant staphylococci. Faecal samples and skin swabs were collected from 166 petted mammals across eight UK centres to recover *E. coli* and coagulase-positive staphylococci (CoPS), respectively, through enrichment culture methods, plating onto non-selective (tryptone bile-x agar, mannitol salt agar) and selective media (ESBL ChromID, mannitol salt agar with 6 mg/L oxacillin). Antimicrobial susceptibility was assessed using Kirby-Bauer disc diffusion, covering eight classes of antimicrobials. Antimicrobial usage records from the past 12-months were obtained from 7/8 centres. Overall, 145/166 faecal samples yielded 223 *E. coli* isolates, with an overall AMR prevalence of 42.6%. Thirteen *E. coli* isolates (from 8.5% of animals) were classified as multidrug-resistant. ESBL-producing *E. coli* were detected in 5/166 faecal samples. From 166 skin swabs, 84 yielded CoPS isolates, with *S. aureus* ( $n = 70$ ), SIG ( $n = 13$ ) and *S. hyicus* ( $n = 1$ ) identified. Overall, 25.3% of SIG isolates exhibited resistance to at least one antimicrobial. Antimicrobial usage correlated positively with AMR prevalence for *E. coli* ( $p < 0.001$ ), though was not associated with multidrug-resistance. This study demonstrates for the first time the presence of AMR within bacteria isolated from UK petting zoo animals, highlighting this reservoir of AMR bacteria.

**Keywords:** antimicrobial resistance; *Staphylococcus aureus*; *Staphylococcus pseudintermedius*; *Escherichia coli*; MRSA; ESBL; petting zoo



Academic Editor: Lucía Fernández

Received: 8 September 2025

Revised: 9 October 2025

Accepted: 14 October 2025

Published: 16 October 2025

**Citation:** Nishigaki, A.; Arden, K.; Frosini, S.-M. Antimicrobial Resistance in Petting Zoo Animals in the United Kingdom. *Appl. Microbiol.* **2025**, *5*, 115. <https://doi.org/10.3390/applmicrobiol5040115>

**Copyright:** © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The rise of antimicrobial resistance (AMR) represents a critical challenge for both human and animal health worldwide. AMR threatens the efficacy of existing antimicrobial therapies and poses a substantial risk to the success of public health and veterinary interventions aimed at controlling bacterial infections. The potential for zoonotic transmission of resistant bacteria underscores the importance of identifying AMR reservoirs in animal populations that frequently interact with humans, such as those in petting zoos and open farms. These environments offer a unique interface where human–animal contact is encouraged, potentially facilitating the transfer of AMR bacteria. This interaction is an example of the One Health concept, which emphasises the interconnectedness of human, animal and

environmental health [1,2]. Of particular concern for spread between human and animal populations over the last twenty years are extended spectrum beta-lactamase-producing (ESBL-) Enterobacteriaceae and methicillin-resistant staphylococci [3–5]. These multidrug-resistant (MDR) pathogens have been implicated in clinical infections in animals but, more critically to the dissemination and transmission of AMR, they can also be carried as part of the normal microbiota of the gastrointestinal tract and skin. Understanding the carriage of MDR organisms and AMR more generally is imperative to discerning and then mitigating the risk of AMR transmission.

ESBL-producing Enterobacteriaceae, such as *Escherichia coli*, are listed by the U.S. Center for Disease Control and Prevention amongst the serious threats to human health [6] and have been frequently identified in domestic animals and livestock [7–9]. ESBL-producing organisms demonstrate resistance to penicillins, cephalosporins (including third generation) and aztreonam [10]. These are also commonly MDR, defined as possessing resistance to at least three classes of antimicrobials [11], usually encompassing tetracyclines, trimethoprim-sulphonamide and fluoroquinolones, amongst other antimicrobial classes. As the genes responsible for ESBLs are often located on plasmids, they can spread amongst bacteria via horizontal gene transfer [12], increasing the risk of resistance dissemination. This ability to transfer resistance complicates infection control efforts, as even non-pathogenic bacteria carrying ESBL genes can act as reservoirs for resistance [13]. Animals colonised with ESBL-producing *E. coli* may not exhibit clinical signs of infection, allowing these resistant strains to persist undetected within petting zoo environments. Humans can be exposed to zoonotic pathogens through oro-faecal transmission after direct interaction with animals or through contaminated surfaces and fomites [14], posing a particular risk to the young, elderly or immunocompromised [15]. In other situations involving close human–animal contact (pet-owning households, livestock workers), transmission of ESBL-producing *E. coli* and other MDR pathogens has been demonstrated [16,17]. Additionally, visitors to petting zoos frequently demonstrate high risk behaviours enabling transmission, such as touching their face or consuming food/beverages without washing their hands after interacting with the animals [18]. While legal requirements mandate that handwashing facilities be provided in these settings, they are often underutilised by visitors, reducing their effectiveness in preventing pathogen transmission [19,20]. Despite confirmation of the transmission of zoonotic pathogens in petting zoos in the United Kingdom (UK) [21,22], AMR prevalence has not been explored. Therefore, potential human exposure to AMR within UK petting zoos, particularly given the reported high-risk behaviours exhibited by visitors, warrants further exploration.

The presence of AMR in *E. coli* derived from zoo animals has been explored by some authors. In a Belgian zoo, De Witte et al. [2] found that 37% ( $n = 14/38$ ) of the sampled zoo mammals showed faecal carriage of ESBL-producing Enterobacteriaceae, with other studies reporting rates of 11–32% ESBL-producing Enterobacteriaceae carriage for zoo animals in Japan, China and the Czech Republic [23–26]. Zoos house a variety of animals, including wild and exotic species, which visitors typically observe from a distance. However, petting zoos are organisations specifically designed for the direct interaction of the public with domesticated or tame animals. AMR has been identified in petting zoos in other countries, with a prevalence of ESBL-producing *E. coli* in animal faecal samples found to be between 2 and 23.9% in Switzerland ( $n = 4/163$ ), Canada ( $n = 21/88$ ) and Israel ( $n = 28/228$ ) [27–29]. However, there has been no exploration of AMR within petting zoo animals in the United Kingdom to date.

Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) are also significant MDR pathogens in human and veterinary medicine, which are resistant to most or all veterinary-licensed antimicrobials [30].

MRSA has been identified in companion animals and livestock, causing skin infections and post-surgical complications [31,32]. Conversely, MRSP is primarily a canine-associated pathogen, where it is an important cause of pyoderma [32,33], and long-term carriage has been reported in dogs following resolution of clinical infection [34,35]. Similarly, carriage of MRSA has also been documented in various animal populations, including livestock [32]. *Staphylococcus pseudintermedius* forms one part of the *Staphylococcus intermedius* group (SIG) alongside *S. intermedius* and *S. delphini*; staphylococci of the SIG group have been detected in clinical infections and carried on the skin and mucosa of numerous other animal species such as cats, pigeons, mink, horses and humans [36]. The complex dynamics of staphylococcal species as transient or resident commensals and opportunistic pathogens between different animal and human hosts is further compounded by description of their ability to share genetic material [37]. Carriage of MDR-staphylococci, therefore, poses a risk for both inter- and cross-species transmission of bacterial isolates and AMR genes, especially where human–animal contact is frequent [38–40]. However, the presence of MDR-staphylococci in petting zoos has been scarcely explored [27,28,41].

To understand carriage of AMR pathogens within petting zoos, this study aimed to identify AMR in *E. coli* and SIG, including selective culture for ESBL-*E. coli* and methicillin-resistant staphylococci, isolated from animal faeces and skin, respectively.

## 2. Materials and Methods

Ethical approval for this study was awarded by the Clinical Research Ethical Review Board at the Royal Veterinary College (URN 2024 2331-A) and received support from the British and Irish Association of Zoos and Aquariums (BIAZA). Petting zoos were included in this study upon providing signed informed consent. For this study, a petting zoo was defined as a facility where members of the public can directly interact with domesticated or semi-domesticated animals. Interaction could include any petting, handling or feeding of animals. Where centres also housed animals not available for interaction with the public, only those available to interact were included in this study. Only mammals were sampled; reptiles and avian species were not included. Mammals were selected opportunistically at the time of sampling, with no specific selection criteria other than their role in human interactions. All available animals were sampled irrespective of age or sex.

Centres were asked to provide records of antimicrobial usage (AMU) in their animals over the previous 12 months. Records could be provided in the form of medicine logbooks or veterinary records. All received records underwent manual review to include only those related to the animal population under study (petting animals), to eliminate antimicrobials not given via systemic administration and to extract data pertaining to antimicrobial class/drug used and the animal species treated. AMU data was categorised at the centre-level to document the number of antimicrobial classes used in the 12-month period and number of individual episodes of use. Episodes were defined as use of (1) a single class of antimicrobial, (2) of any dose, (3) whereby there was less than the expected duration of activity between that dose and the next, as defined by the product datasheet (i.e., continuous dosing).

A single sterile charcoal transport swab was rolled on the animal's skin across multiple sites (head, neck, dorsum, muzzle, nares and buccal mucosa inside the lip, on the outside of the teeth) [42]. In those with a dense fur coat, the swab was positioned as close to the skin as possible before rolling. Faecal samples were collected from each animal group's enclosure floor before daily cleaning. Faecal samples were frozen at  $-80\text{ }^{\circ}\text{C}$ , and skin swabs were immediately processed on receipt at the laboratory (following up to one week at  $4\text{ }^{\circ}\text{C}$  where necessary). Samples were primarily collected by the researcher. In instances where this was not possible, petting zoo staff were provided with written instructions, sampling

materials and transport boxes to enable immediate collection. Samples were then shipped by mail courier directly to the laboratory for analysis and processed on the day of receipt.

For each faecal sample, 1 g of faeces was enriched into 9 mL peptone water (PW; CM0009, Oxoid, Basingstoke, UK) or a corresponding 1:10 faeces (g)/peptone water (mL) where there was less than 1 g of faeces available, and this was incubated at 37 °C for 24 h [43]. Skin swabs were enriched in tryptone soy broth with 10% NaCl (TSB+; CM0129, Oxoid) for 24–48 h at 37 °C, until turbidity was noted [42]. For the detection of *E. coli*, inoculated PW (25 µL) was streaked onto both Tryptone Bile X-glucuronide agar (TBX; CM0945, Oxoid) and ChromID ESBL agar (ChromID; BioMérieux, Basingstoke, UK) and incubated at 37 °C for 24 h. For the detection of *Staphylococcus* spp., inoculated TSB+ (25 µL) was streaked onto both mannitol salt agar (MSA; CM0085, Oxoid) and MSA supplemented with 6 mg/L oxacillin (MSA+) and incubated at 37 °C for 24–48 h. All morphologically distinct, presumptive *E. coli* or staphylococcal colonies were subcultured onto 5% sheep blood agar (CM0854; Oxoid) and incubated at 37 °C for 24 h. Where multiple morphologies on a plate resembled that expected for the bacterial species of interest, each individual morphology was subcultured.

Characteristic colony morphology on 5% sheep blood agar, combined with biochemical testing (catalase-positive, indole-positive and citrate-negative), was used to phenotypically confirm *E. coli*. Suspected ESBL-*E. coli* were further confirmed using MALDI-TOF (VITEK-MS, BioMérieux).

Similarly, coagulase-positive staphylococci were identified through characteristic morphology, differentiated through biochemical testing (catalase-positive, DNase-positive, Voges–Proskauer [VP]-positive *S. aureus* or VP-negative SIG). For further confirmation of staphylococcal species, end-point PCR following DNA extraction (boil lysis) [43] was used, including previously described primers and PCR conditions for *S. aureus* protein A (*spa*) [44] or SIG thermonuclease (*nuc*) [45]. Where genotype did not match the predicted phenotype, MALDI-TOF was used for final CoPS species identification.

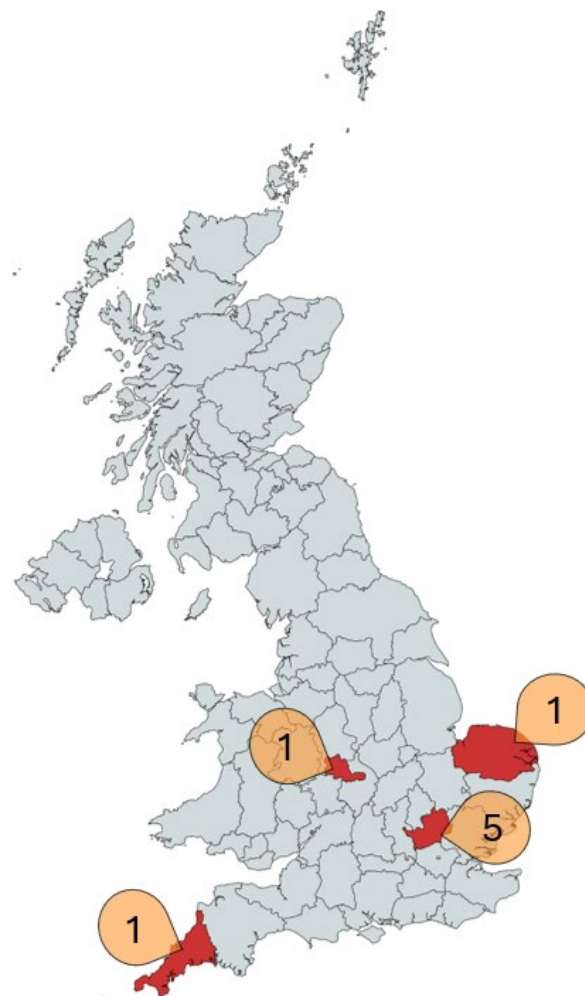
Antimicrobial susceptibility testing (AST) used Kirby–Bauer disc diffusion, following Clinical and Laboratory Standards Institute (CLSI) methods [46]. Isolates were tested against eight antimicrobial classes. For *E. coli*, ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefotaxime or ceftazidime (30 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), gentamicin (10 µg), tetracycline (30 µg) and trimethoprim/sulfamethoxazole (TMPS; 1.25/23.75 µg) were tested (all disks Oxoid). For CoPS, benzylpenicillin (10 µg), cefoxitin (*S. aureus* only; 30 µg), oxacillin (SIG only; 1 µg), chloramphenicol (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), tetracycline (30 µg) and TMPS (1.25/23.75 µg) were tested (all disks Oxoid). Zones of inhibition were interpreted using CLSI-VET01S clinical breakpoints (enrofloxacin, gentamicin, oxacillin, tetracycline and chloramphenicol) [47] or CLSI-M100 breakpoints (for all others) [48]. For all isolates cultured from ESBL ChromID, confirmation of ESBL or Ampicillinase C (AmpC) phenotype was performed using a commercial disk test (D72C, Mast Group, Bootle, UK), as per manufacturer guidelines.

For analysis, ‘intermediate’ interpretation of AST was grouped with ‘resistant’ as ‘non-susceptible’, and MDR was defined as resistance to three or more antimicrobial classes tested [11]. AMR prevalence was calculated from isolates cultured through non-MDR selective agar. A Shapiro–Wilk test assessed normality of distribution, with statistical significance at  $p \leq 0.05$ . All statistical tests were performed in GraphPad Prism version 10.2.3 (GraphPad Software, Boston, MA, USA, [www.graphpad.com](http://www.graphpad.com)). Mann–Whitney U compared CoPS recovery data between the swabs collected by the researcher and those collected by petting zoo staff.

Logistic regression was performed using R (version 4.2.3; RStudio, 2025.05.1+513) to model the prevalence of AMR or MDR in *E. coli* isolates based on categorical predictor values of centre and host animal species. The model was assessed using likelihood ratio tests, with significance at  $p \leq 0.05$ . Furthermore, logistic regression was also used to model the impact of number of AMU episodes and number of antimicrobial classes used at the centre level on AMR and MDR in *E. coli* isolates. Significance of the predictors was assessed using Wald chi-square tests.

### 3. Results

Eight petting zoos representing four regions of the UK were enrolled (Figure 1). Paired skin swabs and faecal samples were collected from 166 animals across the eight centres (Table 1).



**Figure 1.** Map of the United Kingdom, with counties highlighted indicating the locations and numbers representing the total number of recruited petting zoos originating from the indicated county.

**Table 1.** Animal species sampled (skin swabs and faecal samples) from eight petting zoos in the United Kingdom ( $n = 166$ ).

| Animal Species                   | Common Name      | Number Sampled | Number of Different Centres Represented |
|----------------------------------|------------------|----------------|-----------------------------------------|
| <i>Bos taurus</i>                | Cattle           | 31             | 2                                       |
| <i>Capra hircus</i>              | Goat             | 26             | 5                                       |
| <i>Cavia porcellus</i>           | Guinea Pig       | 6              | 3                                       |
| <i>Equus asinus</i>              | Donkey           | 7              | 4                                       |
| <i>Equus caballus</i>            | Horse/Pony       | 15             | 5                                       |
| <i>Hydrochoerus hydrochaeris</i> | Capybara         | 3              | 1                                       |
| <i>Mustela furo</i>              | Ferret           | 6              | 2                                       |
| <i>Oryctolagus cuniculus</i>     | Rabbit           | 14             | 4                                       |
| <i>Ovis aries</i>                | Sheep            | 9              | 3                                       |
| <i>Rangifer tarandus</i>         | Reindeer         | 2              | 1                                       |
| <i>Suricata suricatta</i>        | Meerkat          | 7              | 1                                       |
| <i>Sus domesticus</i>            | Pig              | 12             | 5                                       |
| <i>Varecia rubra</i>             | Red Ruffed Lemur | 3              | 1                                       |
| <i>Vicugna pacos</i>             | Alpaca           | 25             | 4                                       |
| Total                            |                  | 166            |                                         |

### 3.1. Antimicrobial Resistance from Non-MDR-Selective Culture

From TBX agar, 223 *E. coli* isolates were recovered from 145/166 faecal samples (87%). Of those samples that recovered *E. coli*, 49% ( $n = 71/145$ ) yielded a single morphologically distinct colony type, 48% ( $n = 69/145$ ) produced two distinct morphologies, and 3% ( $n = 5/145$ ) produced three.

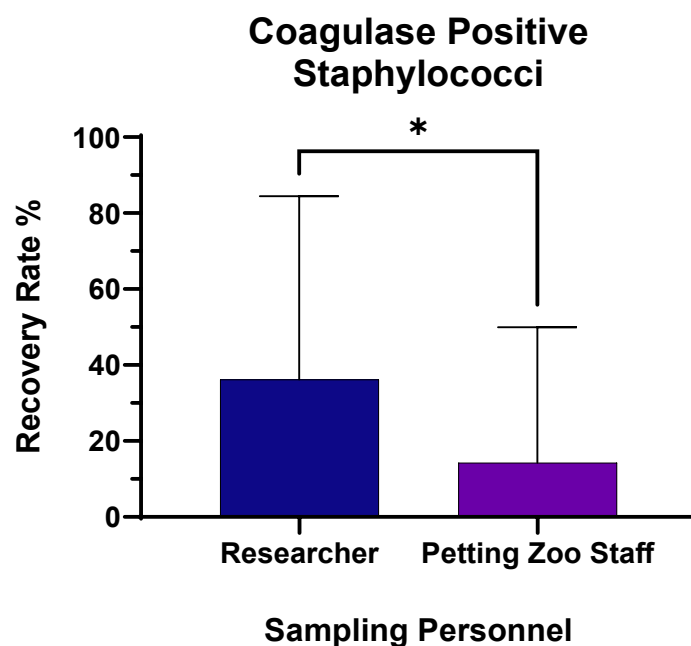
The prevalence of AMR in *E. coli* was 42.6% ( $n = 95/223$ ), encompassing non-susceptibility (intermediate or resistant) to ampicillin ( $n = 78$ , 34.8%); chloramphenicol ( $n = 19$ , 8.5%); enrofloxacin ( $n = 17$ , 7.6%); gentamicin ( $n = 16$ , 7.1%); amoxicillin-clavulanic acid ( $n = 15$ , 6.7%); tetracycline ( $n = 10$ , 4.5%); TMPS ( $n = 9$ ; 4.0%); and third-generation cephalosporins ( $n = 1$ , 0.4%; ceftazidime) (Table 2). MDR prevalence within *E. coli* was 8.5% (Table 2).

From 166 skin swabs,  $n = 54$  (33%) yielded CoPS on MSA. Of the 54 skin swabs yielding CoPS, 56% ( $n = 30/54$ ) yielded one colony morphologically typical of CoPS, 35% ( $n = 19/54$ ) yielded two morphologies, 7% ( $n = 4/54$ ) yielded three, and 2% ( $n = 1/54$ ) yielded four, giving a total of 84 CoPS isolates. CoPS were detected in the swabs from 54 animals, including goats ( $n = 20$ ), alpacas ( $n = 12$ ), sheep ( $n = 6$ ), meerkats ( $n = 6$ ), cows ( $n = 3$ ), capybaras ( $n = 3$ ), donkeys ( $n = 2$ ), horses ( $n = 1$ ) and pigs ( $n = 1$ ) (Supplementary Table S1). Recovery rates of CoPS from skin swabs differed significantly between the samples collected by the researcher (45%) and those collected by petting zoo staff (14%) ( $p = 0.03$ ) (Figure 2) (Supplementary Table S1).

**Table 2.** Antimicrobial resistance profiles following disk diffusion susceptibility testing of 223 *Escherichia coli* isolated from faeces of petting zoo animals ( $n = 166$ ) in the United Kingdom.

|                                     | Number of <i>E. coli</i> (Total $n = 223$ ) | %    |
|-------------------------------------|---------------------------------------------|------|
| <b>No Resistance</b>                | 128                                         | 57.1 |
| <b>Single Resistance</b>            | 54                                          | 24.1 |
| Ampicillin (AMP)                    | 42                                          |      |
| Amoxicillin-clavulanic acid (AMC)   | 1                                           |      |
| Chloramphenicol (C)                 | 5                                           |      |
| Gentamicin (CN)                     | 5                                           |      |
| Enrofloxacin (ENR)                  | 0                                           |      |
| Trimethoprim-sulfamethoxazole (SXT) | 0                                           |      |
| Tetracycline (TE)                   | 1                                           |      |
| 3rd Generation Cephalosporins (3GC) | 0                                           |      |
| <b>Double Resistance</b>            | 22                                          | 9.8  |
| AMP-AMC                             | 2                                           |      |
| AMP-C                               | 4                                           |      |
| AMP-CN                              | 2                                           |      |
| AMP-ENR                             | 5                                           |      |
| AMP-SXT                             | 3                                           |      |
| AMP-TE                              | 2                                           |      |
| C-ENR                               | 1                                           |      |
| ENR-TE                              | 1                                           |      |
| SXT-TE                              | 2                                           |      |
| <b>Multidrug resistance (MDR)</b>   | 19                                          | 8.5  |
| AMP-AMC-C                           | 1                                           |      |
| AMP-AMC-CN                          | 3                                           |      |
| AMP-AMC-ENR                         | 1                                           |      |
| AMP-AMC-SXT                         | 1                                           |      |
| AMP-C-ENR                           | 1                                           |      |
| AMP-C-SXT                           | 1                                           |      |
| AMP-C-TE                            | 1                                           |      |
| AMP-ENR-TE                          | 1                                           |      |
| AMC-CN-ENR                          | 1                                           |      |
| AMP-AMC-C-CN                        | 1                                           |      |
| AMP-AMC-C-3GC                       | 1                                           |      |
| AMP-AMC-CN-ENR                      | 1                                           |      |
| AMP-C-CN-ENR                        | 1                                           |      |
| AMP-ENR-SXT-TE                      | 2                                           |      |
| AMP-AMC-C-CN-ENR                    | 2                                           |      |

Of the 84 identified CoPS isolates, 70 VP-positive isolates were confirmed as *S. aureus*; 13 VP-negative CoPS were confirmed as SIG and a single VP-positive, *spa*-negative isolate was identified by MALDI-TOF as *Staphylococcus hyicus* (identified from an alpaca) (Supplementary Table S1). Simultaneous carriage of *S. aureus* and SIG was found in three animals ( $n = 2$  alpacas,  $n = 1$  meerkat).



**Figure 2.** Recovery of coagulase positive staphylococci from skin swabs of mammals at petting zoos ( $n = 166$ ), collected by the researcher ( $n = 147$ ) versus petting zoo staff ( $n = 19$ ); \* denotes significance ( $p \leq 0.05$ ).

The prevalence of AMR in 83 CoPS isolates (*S. aureus* and SIG combined) was 25.3% ( $n = 21/83$ ) (Table 3). In *S. aureus*, isolates were resistant to enrofloxacin ( $n = 7$ , 10.1%), tetracycline ( $n = 10$ ), erythromycin ( $n = 6$ , 8.7%) and benzylpenicillin ( $n = 1$ , 1.4%) (Table 3). In SIG, AMR was identified in two isolates: one with resistance to benzylpenicillin ( $n = 1$ , 7.7%) alone and the second with an MDR-phenotype displaying resistance to enrofloxacin, erythromycin and tetracycline (Table 3).

**Table 3.** Antimicrobial resistance profiles following disk diffusion susceptibility testing of 83 coagulase-positive staphylococci ( $n = 70$  *Staphylococcus aureus*,  $n = 13$  *Staphylococcus intermedius* group) isolated from skin swabs of petting zoo animals ( $n = 166$ ) in the United Kingdom.

|                                     | <i>Staphylococcus aureus</i> | %    | <i>Staphylococcus intermedius</i> Group (SIG) | %    |
|-------------------------------------|------------------------------|------|-----------------------------------------------|------|
| <b>No Resistance</b>                | 51                           | 72.9 | 11                                            | 84.6 |
| <b>Single Resistance</b>            | 14                           | 20.0 | 1                                             | 7.7  |
| Benzylpenicillin (P)                | 1                            |      | 1                                             |      |
| Cefoxitin (FOX)                     | 0                            |      | N/A                                           |      |
| Oxacillin (OX)                      | N/A                          |      | 0                                             |      |
| Enrofloxacin (ENR)                  | 3                            |      | 0                                             |      |
| Erythromycin (E)                    | 2                            |      | 0                                             |      |
| Chloramphenicol (C)                 | 0                            |      | 0                                             |      |
| Gentamicin (CN)                     | 0                            |      | 0                                             |      |
| Trimethoprim Sulfamethoxazole (SXT) | 0                            |      | 0                                             |      |
| Tetracycline (TE)                   | 8                            |      | 0                                             |      |
| <b>Double Resistance</b>            | 5                            | 7.1  | 0                                             | 0    |
| ENR-E                               | 3                            |      | 0                                             |      |
| ENR-TE                              | 1                            |      | 0                                             |      |
| E-TE                                | 1                            |      | 0                                             |      |
| <b>Multidrug Resistance (MDR)</b>   | 0                            | 0    | 1                                             | 7.7  |
| ENR-E-TE                            | 0                            |      | 1                                             |      |

### 3.2. ESBL-Producing *E. coli*, MRSA and MRSP

Of 166 faecal samples, five yielded ESBL-producing *E. coli* (3%), isolated from ChromID ( $n = 3$  pigs,  $n = 2$  goats, across three different centres). All were confirmed as ESBL-producers through commercial disk testing and so were considered resistant to ampicillin and first to third generation cephalosporins; all were MDR (Table 4). No MRSA or MRSP were detected through selective culture on MSA+.

### 3.3. Effect of Centre and Animal Species on AMR and MDR in *E. coli*

Logistic regression analyses revealed that both centre and host animal species were highly significant predictors of AMR and MDR at the *E. coli* isolate level. Likelihood ratio tests (LRTs) comparing null models to models including each predictor independently indicated a significant improvement in model fit for both centre (AMR:  $\chi^2 = 159.34$ ,  $p < 2.2 \times 10^{-16}$ ; MDR:  $\chi^2 = 70.32$ ,  $p = 3.52 \times 10^{-13}$ ) and animal species (AMR:  $\chi^2 = 559.93$ ,  $p < 2.2 \times 10^{-16}$ ; MDR:  $\chi^2 = 224.69$ ,  $p < 2.2 \times 10^{-16}$ ). When included together, both predictors retained significant effects (all  $p < 2.2 \times 10^{-16}$ ), indicating independent contributions to AMR and MDR risk.

### 3.4. Effect of Antimicrobial Use on AMR and MDR Prevalence in *E. coli*

Data on antimicrobial use was received from 7/8 centres. Four centres had no antimicrobial use documented in the preceding 12 months. In the other three centres, three classes of systemic antimicrobial were used in the preceding 12 months, encompassing tetracyclines ( $n = 2$  centres), aminopenicillins ( $n = 1$  centre), potentiated aminopenicillins ( $n = 1$  centre) and potentiated sulphonamides ( $n = 1$  centre). In a single centre, there were 22 episodes of antimicrobial use in the preceding 12 months in 20 different animals; for the other 2 centres, a single episode of antimicrobial use was recorded in each centre, respectively.

At the centre level, individual logistic regression models demonstrated that both the number of AMU episodes and the number of antimicrobial classes used were significantly associated with resistance prevalence. Each additional AMU episode was associated with a 10.7% increase in the odds of resistance ( $\beta = 0.102$ , OR = 1.11,  $p < 0.001$ ), while each additional antimicrobial class doubled the odds of resistance ( $\beta = 0.702$ , OR = 2.02,  $p < 0.001$ ). In contrast, neither predictor was significantly associated with MDR prevalence when considered individually. In a combined model including both AMU episodes and antimicrobial classes, neither predictor remained statistically significant to predict centre-level AMR or MDR prevalence, despite clear associations in the univariable analyses. This attenuation is likely due to strong collinearity between the two predictors, as evidenced by a Pearson correlation coefficient of  $r = 0.96$ .

**Table 4.** Antimicrobial resistance profiles following disk diffusion susceptibility testing of 5 extended-spectrum beta-lactamase-producing (ESBL-) *Escherichia coli* isolated from faeces of petting zoo animals (*n* = 166) in the United Kingdom.

| Animal Species<br>Originating from | Centre | Ampicillin | Amoxicillin-<br>Clavulanic Acid | 3rd Generation<br>Cephalosporins | Chloramphenicol | Gentamicin | Enrofloxacin | Trimethoprim-<br>Sulphamethoxazole | Tetracycline |
|------------------------------------|--------|------------|---------------------------------|----------------------------------|-----------------|------------|--------------|------------------------------------|--------------|
| Pig                                | A      | R          | S                               | R                                | S               | S          | R            | S                                  | S            |
|                                    | A      | R          | S                               | R                                | S               | S          | R            | S                                  | S            |
|                                    | B      | R          | S                               | R                                | S               | S          | R            | R                                  | R            |
| Goat                               | C      | R          | S                               | R                                | S               | R          | S            | S                                  | S            |
|                                    | C      | R          | R                               | R                                | S               | R          | S            | S                                  | S            |

## 4. Discussion

This study represents the first multi-centric exploration of AMR prevalence in UK petting zoos. The detection of AMR, especially the detection of clinically significant ESBL-*E. coli*, in this population of animals underscores the importance of considering all animals in risk analysis for potential bi-directional sharing of AMR between host species.

Prevalence of AMR in *E. coli* isolates was 42.4%, comparable to that seen previously in petting zoo animals' faeces, where AMR prevalence ranged from 6 to 83%, depending on the species sampled, with a 22% prevalence overall [27]. In the current study, ampicillin was the most frequent antimicrobial to which *E. coli* were resistant (34.8%). This finding aligns with previous studies in livestock and companion animals, where beta-lactam resistance is widespread due to the frequent use of penicillins [49,50], and where ampicillin resistance has been most frequently implicated in pigs, cattle and sheep in the UK (30–73%) [51]. Similarly, in Canadian petting zoo animals, resistance to ampicillin was seen commonly, although tetracycline resistance was more prevalent in that population, potentially relating to geographical differences in antimicrobial use in animal populations [27]. The resistance to second-line antimicrobials, notably enrofloxacin (7.6%) and gentamicin (7.1%), observed in this study, despite no record of use of these antimicrobial classes in the preceding 12 months, is notable. In particular, enrofloxacin resistance was always associated with other drug resistances, highlighting the potential for inadvertent selection of fluoroquinolone resistance when using first-line antimicrobials, which could propagate key AMR profiles without direct selection pressure.

### 4.1. MDR *E. coli*: Prevalence, Diversity and Implications

While MDR was relatively uncommon, its presence in *E. coli* isolates derived from half of the participating centres indicates the risk of emerging resistance in petting zoos. The diversity of MDR profiles suggests the circulation of multiple resistant strains or the acquisition of resistance genes through horizontal gene transfer [52,53]. The detection of 8.5% MDR *E. coli* in this study was at the lower end of rates reported previously in livestock populations across other countries (8–15%) [49,50,54]. This low prevalence may reflect differences in antimicrobial usage in livestock [55], where it is more frequently employed for herd-level metaphylaxis, as compared to petting zoo animals, which are treated on a more individual-animal basis. The difference in AMR across the different centres and host animal species within this study may also highlight the importance that provenance of the animals in petting zoos can have on the presence of key AMR profiles. Many animals in these settings are ex-livestock and thus may have experienced higher selection pressure for AMR than those species that were rescues or were bred within the petting zoo itself. While this study examined the point prevalence of AMR, longitudinal sampling with greater depth of epidemiological evaluation of the animals sampled (e.g., origin, >12-month antimicrobial history) could provide deeper insights into the temporal dynamics and origins of AMR, including the emergence and persistence of MDR. Previous studies in both animals and humans have demonstrated the value of such approaches in capturing fluctuations in resistance patterns over time [56,57].

### 4.2. Patterns of AMU and Resistance

Unlike the current study, Conrad et al. [27] and Isler et al. [28] did not report any AMU data. However, Shnaiderman-Torban et al. [29] did find a significant association between AMU and the shedding of ESBL-Enterobacteriaceae in petting zoo animals. Specifically, the number of antimicrobial classes reportedly used was comparable to that in the current study, with an overlap in the use of potentiated sulphonamides, tetracyclines and amphenicols. However, they reported the use of a greater number of critically important

antimicrobials (CIAs), including cephalosporins, metronidazole and fluoroquinolones [29], none of which were used by the centres in the current study. In livestock, similar results have been found, where a strong correlation has been reported between the use of fluoroquinolones, amphenicols, cephalosporins and sulphonamides and AMR [58]. Overall, it was encouraging to observe low antimicrobial usage across all petting zoos included in this study. Even among the centres that did report antimicrobial use, no prophylactic administration was recorded, suggesting that antimicrobials were used only when deemed necessary for treatment. This trend aligns with responsible antimicrobial stewardship principles and may help mitigate the risk of resistance development [59].

Both centre and animal species emerged as independent predictors of resistance, likely reflecting differences in antimicrobial use practices, husbandry and biosecurity policies across centres, as well as species-level variation in microbiome composition, physiology and exposure pathways. Together, these findings highlight the multifactorial drivers of AMR within petting zoo settings. In parallel, our analysis treated the number of antimicrobial use episodes and the number of antimicrobial classes as separate predictors to aid interpretability; however, their strong collinearity raises the possibility of biased estimates of their individual effects. To address this limitation, future studies could consider alternative approaches, such as penalised regression (e.g., ridge regression, LASSO) or principal component analysis, to better account for the interdependence between these variables.

#### 4.3. Global Differences in Prevalence of ESBL-Producing *E. coli*

The prevalence of ESBL-producing *E. coli* (3%) in this study is comparable to that reported across European petting zoos, namely  $\leq 1\%$  in Germany [41] and 2% in Switzerland [28], but lower than global ESBL-carriage in these centres (12% in Israel [29], 24% in Canada [27]). The methods for recovering ESBL-producing *E. coli* were generally consistent across studies, with enrichment followed by selective media commonly used, although the choice of selective agar varied between studies (CHROMagar ESBL, Brilliance ESBL agar, Biomerieux CHROMID or MacConkey agar supplemented with ceftriaxone, followed by Tryptic Soy Agar containing ampicillin). However, the performances of these media have been shown to be comparable [60,61]. However, Götting et al. [41] collected rectal swabs instead of faecal samples, which may explain the lower detection of ESBL-producing species in their study; rectal swabs have been shown to be less sensitive for the recovery of third-generation cephalosporin-resistant Enterobacterales, compared to enriched faecal samples [62]. The geographic variation in prevalence across studies may reflect differences in AMU practices, husbandry and biosecurity between countries. Geographical factors such as the presence of wildlife reservoirs and regional differences in environmental resistance may also contribute [63]. Further characterization of the ESBL isolates cultured during the process of AMR surveillance, through whole genome sequencing, could, in the future, provide further insight into the transmission dynamics and origins of these MDR pathogens in petting zoos.

The relatively low prevalence of ESBL-producing *E. coli* in this study, as seen across Europe, is encouraging, as it suggests that petting zoos may currently pose a lower risk of transmitting these key MDR pathogens to visitors compared to other sectors where higher prevalence is seen. However, even low-level prevalence warrants consideration, as petting zoos provide frequent opportunities for human–animal interactions, creating a potential pathway for zoonotic transmission, as has been reported in livestock populations [64]. This is particularly concerning, given the clinical importance of ESBL-producing *E. coli* in human and veterinary medicine [65,66]. Consistent with the findings of this study, ESBL-producing *E. coli* have also been identified in pigs and goats in previous petting zoo studies worldwide [27–29,41], indicating potentially higher carriage rates in these species.

However, to date, no studies have specifically investigated preferential carriage of MDR pathogens in these animals within petting zoo settings.

#### 4.4. CoPS AMR and Recovery

AMR in CoPS has been scarcely investigated in the petting zoo population. Although no MDR CoPS isolates were found in this study, the presence of individual resistances to enrofloxacin and erythromycin (both CIAs) deserves attention [52]. These resistances highlight the potential for selective pressure in petting zoos, raising concerns about the spread of AMR and its implications for both animal and public health. Encouragingly, despite the widespread nature of penicillin resistance [67], AMR was infrequently observed in the current study.

It was also reassuring to note that no MRSA or MRSP were detected in this study. Previous studies have explored the presence of MRSA in faecal samples from petting zoo animals [28] and nasal swabs from pigs [27], with the same lack of detection as reported here. In Germany, one caprine MRSA carrier ( $n = 1/300$ ) was identified from a skin swab [41]. Their study included a larger number of animals and centres, although focusing solely on goats, suggesting that larger samples sizes and broader geographic coverage can increase the likelihood of detecting MRSA. The absence of MRSA/MRSP may reflect genuinely low prevalence in UK petting zoos or could be due to study limitations, such as low CoPS recovery from skin swabs. The swabs were stored and processed according to established protocols [42,68,69]. Furthermore, considering evidence of CoPS carriage in the nares of people and animals, as well as in the buccal mucosa [70–72], these sites were chosen for swabbing, and enrichment methods were employed, to maximise the chances of detecting these bacterial species. The multisite sampling method also follows the recommendation for the effective recovery of MRSP in dogs [42]. It is also notable that swabbing the oral cavity and buccal mucosa was intended to account for the possibility of animals licking visitors' hands, particularly in settings where feeding was permitted, as a likely route for bacterial transmission. The lower recovery rate of CoPS from staff-collected compared to researcher-collected samples underscores the importance of sampling methodology in AMR surveillance studies. This difference may reflect variation in the confidence and consistency of sampling techniques, as researchers with greater background knowledge may be more likely to target anatomical sites where CoPS carriage is concentrated (e.g., buccal mucosa, nares). While these factors do not invalidate the findings, they may partly explain the reduced recovery from staff-collected samples and highlight the need for improved instruction or direct researcher collection in future studies. Ensuring appropriate and consistent sample collection is crucial for obtaining accurate AMR prevalence data, as inconsistencies could lead to underestimation of bacterial presence, including MDR organisms such as MRSA and MRSP. In addition, this study did not include avian or reptile species commonly found in petting zoos. Consequently, the prevalence and patterns of AMR reported here may not fully reflect the broader diversity of organisms circulating in these settings. Future studies incorporating these taxa would provide a more comprehensive understanding of AMR dynamics across all animals in petting zoo environments.

The recovery of CoPS overall remained notably lower in this study (33%) than the 53–89% recovery rates reported in other domesticated animals [73–76]. This disparity may be attributed to several factors, including inherent differences in the skin microbiome of the sampled animals. The composition and diversity of the skin microbiome can vary between individuals and species, potentially influencing the prevalence of specific bacterial populations, such as CoPS [77]. Additionally, interactions between CoPS and coagulase-negative staphylococci (CoNS) may further influence these differences, as CoNS have been shown to inhibit *S. aureus* colonisation in people [78,79]. This highlights the dynamic nature

of the skin microbiome and its impact on recovery of bacterial species. However, as CoNS can act as reservoirs for *mecA* and other resistance genes that could then be transmitted to CoPS [80], broader surveillance through metagenomic methods could highlight key AMR reservoirs currently not described.

## 5. Conclusions

Outbreaks of *E. coli* O157:H7 in petting zoos have been reported worldwide [21,81,82], along with other pathogens, such as *Salmonella* spp., *Campylobacter* spp. and *Cryptosporidium* spp. [82]. Cases of zoonotic transmission of bacteria underscore the importance of understanding the risk of transmission of AMR bacteria in petting zoo environments. Given the frequent interactions between humans and animals in petting zoos, including direct contact with animal faeces, contaminated surfaces and animals themselves, the risk of zoonotic transmission is elevated [18]; this risk is complicated when AMR bacteria are present. Additionally, the potential for horizontal gene transfer within bacterial communities in petting zoo environments, where diverse animal species interact, could enhance the spread of AMR among animals [83] and potentially to people in close proximity. The emergence and spread of AMR pathogens pose a greater threat to vulnerable populations, such as young children, the elderly and immunocompromised individuals. Effective hygiene practices, such as handwashing stations and educating the public on the risks of handling animals are essential to reducing the likelihood of zoonotic transmission of pathogens [84].

This study demonstrates for the first time the presence of AMR *E. coli* and *Staphylococcus* spp. in UK petting zoo animals, with significant variation in resistance prevalence across different centres. The detection of ESBL-producing *E. coli* highlights the public health risks associated with potential zoonotic transmission of clinically significant AMR bacteria, emphasising the need for heightened vigilance in these settings. The strong correlation between antimicrobial use and overall resistance prevalence further emphasises the critical role of responsible antimicrobial stewardship in animal husbandry. Future studies should incorporate molecular characterisation of resistance genes, larger sample sizes and longitudinal sampling to better understand temporal patterns, including seasonal fluctuations, persistence and the emergence of antimicrobial resistance within individual animals, groups and across petting zoo sites. Whole-genome sequencing and metagenomics could be employed to comprehensively characterise the diversity of resistance genes and microbial communities, providing insight into the mechanisms and mobility of resistance determinants. Investigating environmental contamination and potential transmission routes would further clarify the drivers of AMR spread and associated public health risks. Complementary studies of visitor compliance with hygiene measures would provide additional context for understanding how human behaviour may influence the transmission of resistant bacteria in these settings.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/applmicrobiol5040115/s1>; Table S1: Recovery of coagulase-positive staphylococci from animals ( $n = 166$ ) housed in eight petting zoos in the United Kingdom.

**Author Contributions:** Conceptualization, A.N., S.-M.F. and K.A.; methodology, S.-M.F.; formal analysis, A.N. and S.-M.F.; investigation, A.N.; data curation, A.N.; writing—original draft preparation, A.N.; writing—review and editing, S.-M.F. and K.A.; supervision, S.-M.F. and K.A.; project administration, A.N.; funding acquisition, A.N. and S.-M.F. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by an MSD Animal Health Research Bursary for Veterinary Students, awarded to A.N.

**Data Availability Statement:** The original contributions presented in this study are included in the article/Supplementary Material. Further discrimination of datasets presented in this article to the level of individual centres are not available because ethical approval required anonymisation of animal- and centre-specific AMR data to protect the interests of collaborating centres. Further inquiries can be directed to the corresponding author(s).

**Acknowledgments:** The authors warmly thank the staff and animals at All Things Wild, Honeybourne; Homerswood Farm Experiences, Welwyn; Meadowfield Alpacas, St Albans; Pets Corner, Harlow; and all other participating centres for their cooperation and support throughout this study. We also thank the British and Irish Association of Zoos and Aquariums for their support of this study.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## Abbreviations

The following abbreviations are used in this manuscript:

|           |                                                              |
|-----------|--------------------------------------------------------------|
| AmpC      | Ampicillinase C                                              |
| AMR       | Antimicrobial resistance                                     |
| AMU       | Antimicrobial use                                            |
| AST       | Antimicrobial susceptibility testing                         |
| BIAZA     | British and Irish Association of Zoos and Aquariums          |
| CIA       | Critically important antimicrobial                           |
| CLSI      | Clinical and Laboratory Standards Institute                  |
| CoNS      | Coagulase-negative staphylococci                             |
| CoPS      | Coagulase-positive staphylococci                             |
| DNA       | Deoxyribonucleic acid                                        |
| ESBL      | Extended-spectrum beta-lactamase                             |
| MALDI-TOF | Matrix assisted laser desorption/ionisation time-of-flight   |
| MDR       | Multidrug-resistant                                          |
| MRSA      | Methicillin-resistant <i>Staphylococcus aureus</i>           |
| MRSP      | Methicillin-resistant <i>Staphylococcus pseudintermedius</i> |
| MSA       | Mannitol salt agar                                           |
| MSA+      | Mannitol salt agar supplemented with 6 mg/L oxacillin        |
| PCR       | Polymerase chain reaction                                    |
| PW        | Peptone water                                                |
| SIG       | <i>Staphylococcus intermedius</i> group                      |
| TBX       | Tryptone bile X-glucuronide agar                             |
| TMPS      | Trimethoprim sulfamethoxazole                                |
| TSB+      | Tryptic soy broth supplemented with 10% Sodium Chloride      |
| UK        | United Kingdom                                               |

## References

1. Velazquez-Meza, M.E.; Galarde-López, M.; Carrillo-Quiróz, B.; Alpuche-Aranda, C.M. Antimicrobial resistance: One Health approach. *Vet. World* **2022**, *15*, 743–749. [[PubMed](#)]
2. De Witte, C.; Vereecke, N.; Theuns, S.; De Ruyck, C.; Vercammen, F.; Bouts, T.; Boyen, F.; Nauwynck, H.; Haesebrouck, F. Presence of Broad-Spectrum Beta-Lactamase-Producing Enterobacteriaceae in Zoo Mammals. *Microorganisms* **2021**, *9*, 834. [[PubMed](#)]
3. Weese, J.S. Methicillin-resistant *Staphylococcus aureus* in animals. *ILAR J.* **2010**, *51*, 233–244. [[CrossRef](#)]
4. Köck, R.; Herr, C.; Kreienbrock, L.; Schwarz, S.; Tenhagen, B.A.; Walther, B. Multiresistant Gram-Negative Pathogens—A Zoonotic Problem. *Dtsch. Arztebl. Int.* **2021**, *118*, 579–589.
5. Bassiouny, M.; Neubauer, H.; Sprague, L.D. Gram-positive ESKAPE pathogens in Germany: A comprehensive analysis of occurrence and resistance development in animal, food, and environmental sources. *One Health* **2025**, *20*, 101099. [[CrossRef](#)]
6. CDC. *Antibiotic Resistance Threats in the United States, 2019*; U.S. Department of Health and Human Services: Atlanta, GA, USA, 2019.

7. Zogg, A.L.; Simmen, S.; Zurfluh, K.; Stephan, R.; Schmitt, S.N.; Nüesch-Inderbilen, M. High Prevalence of Extended-Spectrum  $\beta$ -Lactamase Producing Enterobacteriaceae Among Clinical Isolates from Cats and Dogs Admitted to a Veterinary Hospital in Switzerland. *Front. Vet. Sci.* **2018**, *5*, 62.
8. Ortiz-Díez, G.; Mengíbar, R.L.; Turrientes, M.-C.; Artigao, M.-R.B.; Gallifa, R.L.; Tello, A.M.; Pérez, C.F.; Santiago, T.A. Prevalence, incidence and risk factors for acquisition and colonization of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae from dogs attended at a veterinary hospital in Spain. *Comp. Immunol. Microbiol. Infect. Dis.* **2023**, *92*, 101922. [[CrossRef](#)]
9. Mandujano-Hernández, A.; Martínez-Vázquez, A.V.; Paz-González, A.D.; Herrera-Mayorga, V.; Sánchez-Sánchez, M.; Lara-Ramírez, E.E.; Vázquez, K.; De Jesús De Luna-Santillana, E.; Bocanegra-García, V.; Rivera, G. The Global Rise of ESBL-Producing *Escherichia coli* in the Livestock Sector: A Five-Year Overview. *Animals* **2024**, *14*, 2490. [[CrossRef](#)]
10. Rawat, D.; Nair, D. Extended-spectrum  $\beta$ -lactamases in Gram Negative Bacteria. *J. Glob. Infect. Dis.* **2010**, *2*, 263–274.
11. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [[CrossRef](#)]
12. Gekenidis, M.-T.; Kläui, A.; Smalla, K.; Drissner, D. Transferable Extended-Spectrum  $\beta$ -Lactamase (ESBL) Plasmids in *Enterobacteriaceae* from Irrigation Water. *Microorganisms* **2020**, *8*, 978. [[CrossRef](#)]
13. Carattoli, A. Plasmids and the spread of resistance. *Int. J. Med. Microbiol.* **2013**, *303*, 298–304. [[CrossRef](#)] [[PubMed](#)]
14. Conrad, C.C.; Stanford, K.; Narvaez-Bravo, C.; Callaway, T.; Mcallister, T. Farm Fairs and Petting Zoos: A Review of Animal Contact as a Source of Zoonotic Enteric Disease. *Foodborne Pathog. Dis.* **2016**, *14*, 59–73. [[CrossRef](#)] [[PubMed](#)]
15. Wise, J. Outbreak of *E. coli* O157 is linked to Surrey open farm. *BMJ* **2009**, *339*, b3795. [[CrossRef](#)]
16. Ljungquist, O.; Ljungquist, D.; Myrenäs, M.; Rydén, C.; Finn, M.; Bengtsson, B. Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs—A pilot study. *Infect. Ecol. Epidemiol.* **2016**, *6*, 31514. [[CrossRef](#)]
17. Van Hoek, A.; Dierikx, C.; Bosch, T.; Schouls, L.; Van Duijkeren, E.; Visser, M. Transmission of ESBL-producing *Escherichia coli* between broilers and humans on broiler farms. *J. Antimicrob. Chemother.* **2020**, *75*, 543–549. [[CrossRef](#)]
18. Weese, J.S.; McCarthy, L.; Mossop, M.; Martin, H.; Lefebvre, S. Observation of Practices at Petting Zoos and the Potential Impact on Zoonotic Disease Transmission. *Clin. Infect. Dis.* **2007**, *45*, 10–15. [[CrossRef](#)]
19. Werden, K.E.B.; Paul, C. Compliance with Hygiene Recommendations for Human-animal Contact at Petting Zoos. *Mich. J. Public Health* **2008**, *2*, 19.
20. Anderson, M.E.C.; Weese, J.S. Video observation of hand hygiene practices at a petting zoo and the impact of hand hygiene interventions. *Epidemiol. Infect.* **2012**, *140*, 182–190.
21. Ihekweazu, C.; Carroll, K.; Adak, B.; Smith, G.; Pritchard, G.C.; Gillespie, I.A.; Verlander, N.Q.; Harvey-Vince, L.; Reacher, M.; Edeghere, O.; et al. Large outbreak of verocytotoxin-producing *Escherichia coli* O157 infection in visitors to a petting farm in South East England, 2009. *Epidemiol. Infect.* **2012**, *140*, 1400–1413. [[CrossRef](#)]
22. Rowell, S.; King, C.; Jenkins, C.; Dallman, T.J.; Decraene, V.; Lamden, K.; Howard, A.; Featherstone, C.A.; Cleary, P. An outbreak of Shiga toxin-producing *Escherichia coli* serogroup O157 linked to a lamb-feeding event. *Epidemiol. Infect.* **2016**, *144*, 2494–2500. [[CrossRef](#)] [[PubMed](#)]
23. Ahmed, A.M.; Motoi, Y.; Sato, M.; Maruyama, A.; Watanabe, H.; Fukumoto, Y.; Shimamoto, T. Zoo animals as reservoirs of gram-negative bacteria harboring integrons and antimicrobial resistance genes. *Appl. Environ. Microbiol.* **2007**, *73*, 6686–6690. [[CrossRef](#)] [[PubMed](#)]
24. Ishihara, K.; Hosokawa, Y.; Makita, K.; Noda, J.; Ueno, H.; Muramatsu, Y.; Mukai, T.; Yamamoto, H.; Ito, M.; Tamura, Y. Factors associated with antimicrobial-resistant *Escherichia coli* in zoo animals. *Res. Vet. Sci.* **2012**, *93*, 574–580. [[CrossRef](#)]
25. Wang, Y.; He, T.; Han, J.; Wang, J.; Foley, S.L.; Yang, G.; Wan, S.; Shen, J.; Wu, C. Prevalence of ESBLs and PMQR genes in fecal *Escherichia coli* isolated from the non-human primates in six zoos in China. *Vet. Microbiol.* **2012**, *159*, 53–59. [[CrossRef](#)]
26. Dobiasova, H.; Dolejska, M.; Jamborova, I.; Brhelova, E.; Blazkova, L.; Papousek, I.; Kozlova, M.; Klimes, J.; Cizek, A.; Literak, I. Extended spectrum beta-lactamase and fluoroquinolone resistance genes and plasmids among *Escherichia coli* isolates from zoo animals, Czech Republic. *FEMS Microbiol. Ecol.* **2013**, *85*, 604–611. [[CrossRef](#)]
27. Conrad, C.C.; Stanford, K.; Narvaez-Bravo, C.; Neumann, N.F.; Munns, K.; Tymensen, L.; Jokinen, C.; Mcallister, T.A. Zoonotic Fecal Pathogens and Antimicrobial Resistance in Canadian Petting Zoos. *Microorganisms* **2018**, *6*, 70. [[CrossRef](#)]
28. Isler, M.; Wissmann, R.; Morach, M.; Zurfluh, K.; Stephan, R.; Nüesch-Inderbilen, M. Animal petting zoos as sources of Shiga toxin-producing *Escherichia coli*, *Salmonella* and extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae. *Zoonoses Public Health* **2021**, *68*, 79–87. [[CrossRef](#)]
29. Shnaiderman-Torban, A.; Steinman, A.; Meidan, G.; Paitan, Y.; Abu Ahmad, W.; Navon-Venezia, S. Petting Zoo Animals as an Emerging Reservoir of Extended-Spectrum  $\beta$ -Lactamase and AmpC-Producing Enterobacteriaceae. *Front. Microbiol.* **2019**, *10*, 2488. [[CrossRef](#)]

30. Morris, D.O.; Loeffler, A.; Davis, M.F.; Guardabassi, L.; Weese, J.S. Recommendations for approaches to methicillin-resistant staphylococcal infections of small animals: Diagnosis, therapeutic considerations and preventative measures: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. *Vet. Dermatol.* **2017**, *28*, 304–e69. [\[CrossRef\]](#)
31. Baptiste, K.E.; Williams, K.; Willams, N.J.; Wattret, A.; Clegg, P.D.; Dawson, S.; Corkill, J.E.; O'Neill, T.; Hart, C.A. Methicillin-resistant staphylococci in companion animals. *Emerg. Infect. Dis.* **2005**, *11*, 1942–1944. [\[CrossRef\]](#)
32. Weese, J.S.; Van Duijkeren, E. Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. *Vet. Microbiol.* **2010**, *140*, 418–429. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Perreten, V.; Kadlec, K.; Schwarz, S.; Grönlund Andersson, U.; Finn, M.; Greko, C.; Moodley, A.; Kania, S.A.; Frank, L.A.; Bemis, D.A.; et al. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: An international multicentre study. *J. Antimicrob. Chemother.* **2010**, *65*, 1145–1154. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Windahl, U.; Reimegård, E.; Holst, B.S.; Egenvall, A.; Fernström, L.; Fredriksson, M.; Trowald-Wigh, G.; Andersson, U.G. Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in dogs—a longitudinal study. *BMC Vet. Res.* **2012**, *8*, 34. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Frosini, S.M.; Bond, R.; King, R.; Feudi, C.; Schwarz, S.; Loeffler, A. Effect of topical antimicrobial therapy and household cleaning on methicillin-resistant *Staphylococcus pseudintermedius* carriage in dogs. *Vet. Rec.* **2022**, *190*, e937.
36. Sasaki, T.; Kikuchi, K.; Tanaka, Y.; Takahashi, N.; Kamata, S.; Hiramatsu, K. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. *J. Clin. Microbiol.* **2007**, *45*, 2770–2778. [\[CrossRef\]](#)
37. Frosini, S.M.; Bond, R.; McCarthy, A.J.; Feudi, C.; Schwarz, S.; Lindsay, J.A.; Loeffler, A. Genes on the Move: In Vitro Transduction of Antimicrobial Resistance Genes between Human and Canine Staphylococcal Pathogens. *Microorganisms* **2020**, *8*, 2031. [\[CrossRef\]](#)
38. Graveland, H.; Duim, B.; Van Duijkeren, E.; Heederik, D.; Wagenaar, J.A. Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. *Int. J. Med. Microbiol.* **2011**, *301*, 630–634.
39. Graveland, H.; Wagenaar, J.A.; Heesterbeek, H.; Mevius, D.; Van Duijkeren, E.; Heederik, D. Methicillin Resistant *Staphylococcus aureus* ST398 in Veal Calf Farming: Human MRSA Carriage Related with Animal Antimicrobial Usage and Farm Hygiene. *PLoS ONE* **2010**, *5*, e10990.
40. Moses, I.B.; Santos, F.F.; Gales, A.C. Human Colonization and Infection by *Staphylococcus pseudintermedius*: An Emerging and Underestimated Zoonotic Pathogen. *Microorganisms* **2023**, *11*, 581. [\[CrossRef\]](#)
41. Götting, J.; Heckel, J.-O.; Hotzel, H.; Fruth, A.; Pfeifer, Y.; Henning, K.; Kopp, P.; Mertens-Scholz, K.; Rietschel, W.; Pfeffer, M. Zoonotic bacteria in clinically healthy goats in petting zoo settings of zoological gardens in Germany. *Zoonoses Public Health* **2022**, *69*, 333–343. [\[CrossRef\]](#)
42. Frosini, S.-M.; Bond, R.; King, R.H.; Loeffler, A. The nose is not enough: Multi-site sampling is best for MRSP detection in dogs and households. *Vet. Dermatol.* **2022**, *33*, 576–580. [\[CrossRef\]](#)
43. Menezes, J.; Frosini, S.M.; Belas, A.; Marques, C.; Da Silva, J.M.; Amaral, A.J.; Loeffler, A.; Pomba, C. Longitudinal study of ESBL/AmpC-producing Enterobacterales strains sharing between cohabiting healthy companion animals and humans in Portugal and in the United Kingdom. *Eur. J. Clin. Microbiol. Infect. Dis.* **2023**, *42*, 1011–1024. [\[CrossRef\]](#)
44. Stegger, M.; Andersen, P.S.; Kearns, A.; Pichon, B.; Holmes, M.A.; Edwards, G.; Laurent, F.; Teale, C.; Skov, R.; Larsen, A.R. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecALGA251*. *Clin. Microbiol. Infect.* **2012**, *18*, 395–400. [\[CrossRef\]](#)
45. Becker, K.; Von Eiff, C.; Keller, B.; Brück, M.; Etienne, J.; Peters, G. Thermonuclease gene as a target for specific identification of *Staphylococcus intermedius* isolates: Use of a PCR-DNA enzyme immunoassay. *Diagn. Microbiol. Infect. Dis.* **2005**, *51*, 237–244. [\[CrossRef\]](#) [\[PubMed\]](#)
46. M02-A13; Performance Standards for Antimicrobial Disk Susceptibility Tests. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2024.
47. VET01S-ED7; Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2024.
48. M100-ED35; 2025 Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2025.
49. Gameda, B.A.; Wieland, B.; Alemayehu, G.; Knight-Jones, T.J.D.; Wodajo, H.D.; Tefera, M.; Kumbe, A.; Olani, A.; Abera, S.; Amenu, K. Antimicrobial Resistance of *Escherichia coli* Isolates from Livestock and the Environment in Extensive Smallholder Livestock Production Systems in Ethiopia. *Antibiotics* **2023**, *12*, 941. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Garzon, A.; Portillo, R.; Habing, G.; Silva-Del-Rio, N.; Karle, B.M.; Pereira, R.V. Antimicrobial resistance of *Escherichia coli* from dairy farms participating in an antimicrobial stewardship educational program for farm employees. *J. Dairy Sci.* **2024**, *107*, 1645–1655. [\[CrossRef\]](#) [\[PubMed\]](#)
51. UK-VARSS 2023; Veterinary Antibiotic Resistance and Sales Surveillance Report (UK-VARSS 2023). Veterinary Medicines Directorate: Addlestone, UK, 2023.

52. Harada, K.; Asai, T. Role of Antimicrobial Selective Pressure and Secondary Factors on Antimicrobial Resistance Prevalence in *Escherichia coli* from Food-Producing Animals in Japan. *BioMed Res. Int.* **2010**, *2010*, 180682.
53. Fletcher, S. Understanding the contribution of environmental factors in the spread of antimicrobial resistance. *Environ. Health Prev. Med.* **2015**, *20*, 243–252.
54. Massé, J.; Lardé, H.; Fairbrother, J.M.; Roy, J.-P.; Francoz, D.; Dufour, S.; Archambault, M. Prevalence of Antimicrobial Resistance and Characteristics of *Escherichia coli* Isolates from Fecal and Manure Pit Samples on Dairy Farms in the Province of Québec, Canada. *Front. Vet. Sci.* **2021**, *8*, 654125.
55. Ardakani, Z.; Aragrande, M.; Canali, M. Global antimicrobial use in livestock farming: An estimate for cattle, chickens, and pigs. *Animal* **2024**, *18*, 101060. [\[CrossRef\]](#)
56. Alali, W.Q.; Scott, H.; Harvey, R.; Norby, B.; Lawhorn, D.; Pillai, S. Longitudinal study of antimicrobial resistance among *Escherichia coli* isolates from integrated multisite cohorts of humans and swine. *Appl. Environ. Microbiol.* **2008**, *74*, 3672–3681. [\[CrossRef\]](#)
57. Liu, C.; Wang, P.; Dai, Y.; Liu, Y.; Song, Y.; Yu, L.; Feng, C.; Liu, M.; Xie, Z.; Shang, Y.; et al. Longitudinal monitoring of multidrug resistance in *Escherichia coli* on broiler chicken fattening farms in Shandong, China. *Poult. Sci.* **2021**, *100*, 100887. [\[CrossRef\]](#)
58. Chantziaras, I.; Boyen, F.; Callens, B.; Dewulf, J. Correlation between veterinary antimicrobial use and antimicrobial resistance in food-producing animals: A report on seven countries. *J. Antimicrob. Chemother.* **2013**, *69*, 827–834. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Dyar, O.J.; Huttner, B.; Schouten, J.; Pulcini, C. What is antimicrobial stewardship? *Clin. Microbiol. Infect.* **2017**, *23*, 793–798. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Willems, E.; Cartuyvels, R.; Magerman, K.; Verhaegen, J. Evaluation of 3 different agar media for rapid detection of extended-spectrum  $\beta$ -lactamase-producing Enterobacteriaceae from surveillance samples. *Diagn. Microbiol. Infect. Dis.* **2013**, *76*, 16–19. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Blane, B.; Brodrick, H.J.; Gouliouris, T.; Ambridge, K.E.; Kidney, A.D.; Ludden, C.M.; Limmathurotsakul, D.; Török, M.E.; Peacock, S.J. Comparison of 2 chromogenic media for the detection of extended-spectrum  $\beta$ -lactamase producing Enterobacteriaceae stool carriage in nursing home residents. *Diagn. Microbiol. Infect. Dis.* **2016**, *84*, 181–183.
62. Jazmati, T.; Hamprecht, A.; Jazmati, N. Comparison of stool samples and rectal swabs with and without pre-enrichment for the detection of third-generation cephalosporin-resistant Enterobacterales (3GCRES). *Eur. J. Clin. Microbiol. Infect. Dis.* **2021**, *40*, 2431–2436.
63. Woolhouse, M.E.; Ward, M.J. Sources of antimicrobial resistance. *Science* **2013**, *341*, 1460–1461. [\[CrossRef\]](#)
64. Nüesch-Inderbinen, M.; Stephan, R. Epidemiology of Extended-Spectrum  $\beta$ -Lactamase-Producing *Escherichia coli* in the Human-Livestock Environment. *Curr. Clin. Microbiol. Rep.* **2016**, *3*, 1–9. [\[CrossRef\]](#)
65. Nóbrega, D.B.; Brocchi, M. An overview of extended-spectrum beta-lactamases in veterinary medicine and their public health consequences. *J. Infect. Dev. Ctries.* **2014**, *8*, 954–960. [\[CrossRef\]](#)
66. Husna, A.; Rahman, M.M.; Badruzzaman, A.T.M.; Sikder, M.H.; Islam, M.R.; Rahman, M.T.; Alam, J.; Ashour, H.M. Extended-Spectrum  $\beta$ -Lactamases (ESBL): Challenges and Opportunities. *Biomedicines* **2023**, *11*, 2937. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Vestergaard, M.; Frees, D.; Ingmer, H. Antibiotic Resistance and the MRSA Problem. *Microbiol. Spectr.* **2019**, *7*, 10.1128. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Delacour, H.; Van Cuyck, H.; Dubrous, P.; Soullié, B.; Leroy, P.; Koeck, J.L. Efficacy of a swab transport system in maintaining long-term viability of *Staphylococcus aureus*. *Diagn. Microbiol. Infect. Dis.* **2009**, *65*, 345–346. [\[CrossRef\]](#)
69. Panisello Yagüe, D.; Mihaljevic, J.; Mbegbu, M.; Wood, C.V.; Hepp, C.; Kyman, S.; Hornstra, H.; Trotter, R.; Cope, E.; Pearson, T. Survival of *Staphylococcus aureus* on sampling swabs stored at different temperatures. *J. Appl. Microbiol.* **2021**, *131*, 1030–1038. [\[CrossRef\]](#)
70. Iverson, S.A.; Brazil, A.M.; Ferguson, J.M.; Nelson, K.; Lautenbach, E.; Rankin, S.C.; Morris, D.O.; Davis, M.F. Anatomical patterns of colonization of pets with staphylococcal species in homes of people with methicillin-resistant *Staphylococcus aureus* (MRSA) skin or soft tissue infection (SSTI). *Vet. Microbiol.* **2015**, *176*, 202–208. [\[CrossRef\]](#)
71. Verstappen, K.M.; Willems, E.; Fluit, A.C.; Duim, B.; Martens, M.; Wagenaar, J.A. *Staphylococcus aureus* Nasal Colonization Differs among Pig Lineages and Is Associated with the Presence of Other Staphylococcal Species. *Front. Vet. Sci.* **2017**, *4*, 97. [\[CrossRef\]](#)
72. Bullone, M.; Bellato, A.; Robino, P.; Nebbia, P.; Morello, S.; Marchis, D.; Tarducci, A.; Ru, G. Prevalence and risk factors associated with nasal carriage of methicillin-resistant staphylococci in horses and their caregivers. *Vet. Res.* **2024**, *55*, 108. [\[CrossRef\]](#)
73. Beça, N.; Bessa, L.J.; Mendes, Â.; Santos, J.; Leite-Martins, L.; Matos, A.J.F.; Da Costa, P.M. Coagulase-Positive *Staphylococcus*: Prevalence and Antimicrobial Resistance. *J. Am. Anim. Hosp. Assoc.* **2015**, *51*, 365–371. [\[CrossRef\]](#)
74. Bean, D.; Wigmore, S. Carriage rate and antibiotic susceptibility of coagulase-positive staphylococci isolated from healthy dogs in Victoria, Australia. *Aust. Vet. J.* **2016**, *94*, 456–460. [\[CrossRef\]](#)
75. Saputra, S.; Jordan, D.; Worthing, K.A.; Norris, J.M.; Wong, H.S.; Abraham, R.; Trott, D.J.; Abraham, S. Antimicrobial resistance in coagulase-positive staphylococci isolated from companion animals in Australia: A one year study. *PLoS ONE* **2017**, *12*, e0176379. [\[CrossRef\]](#)

76. Elmoslemany, A.; Elsohaby, I.; Alorabi, M.; Alkafafy, M.; Al-Marri, T.; Aldoweriej, A.; Alaql, F.A.; Almubarak, A.; Fayed, M. Diversity and Risk Factors Associated with Multidrug and Methicillin-Resistant Staphylococci Isolated from Cats Admitted to a Veterinary Clinic in Eastern Province, Saudi Arabia. *Antibiotics* **2021**, *10*, 367. [[CrossRef](#)] [[PubMed](#)]
77. Ross, A.A.; Rodrigues Hoffmann, A.; Neufeld, J.D. The skin microbiome of vertebrates. *Microbiome* **2019**, *7*, 79. [[CrossRef](#)] [[PubMed](#)]
78. Nakatsuji, T.; Chen, T.H.; Narala, S.; Chun, K.A.; Two, A.M.; Yun, T.; Shafiq, F.; Kotol, P.F.; Bouslimani, A.; Melnik, A.V.; et al. Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis. *Sci. Transl. Med.* **2017**, *9*, eaah4680. [[CrossRef](#)] [[PubMed](#)]
79. Paharik, A.E.; Parlet, C.P.; Chung, N.; Todd, D.A.; Rodriguez, E.I.; Van Dyke, M.J.; Cech, N.B.; Horswill, A.R. Coagulase-Negative Staphylococcal Strain Prevents *Staphylococcus aureus* Colonization and Skin Infection by Blocking Quorum Sensing. *Cell Host Microbe* **2017**, *22*, 746–756.e5. [[CrossRef](#)]
80. Tsubakishita, S.; Kuwahara-Arai, K.; Sasaki, T.; Hiramatsu, K. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. *Antimicrob. Agents Chemother.* **2010**, *54*, 4352–4359. [[CrossRef](#)]
81. Warshawsky, B.; Gutmanis, I.; Henry, B.; Dow, J.; Reffle, J.; Pollett, G.; Ahmed, R.; Aldom, J.; Alves, D.; Chagla, A.; et al. Outbreak of *Escherichia coli* O157:H7 related to animal contact at a petting zoo. *Can. J. Infect. Dis. Med. Microbiol.* **2002**, *13*, 175–181. [[CrossRef](#)]
82. Schlager, S.; Lepuschitz, S.; Ruppitsch, W.; Ableitner, O.; Pietzka, A.; Neubauer, S.; Stöger, A.; Lassnig, H.; Mikula, C.; Springer, B.; et al. Petting zoos as sources of Shiga toxin-producing *Escherichia coli* (STEC) infections. *Int. J. Med. Microbiol.* **2018**, *308*, 927–932. [[CrossRef](#)]
83. Dunn, J.R.; Behraves, C.B.; Angulo, F.J. Diseases Transmitted by Domestic Livestock: Perils of the Petting Zoo. *Microbiol. Spectr.* **2015**, *3*. [[CrossRef](#)]
84. Bloomfield, S.F.; Aiello, A.E.; Cookson, B.; O’boyle, C.; Larson, E.L. The effectiveness of hand hygiene procedures in reducing the risks of infections in home and community settings including handwashing and alcohol-based hand sanitizers. *Am. J. Infect. Control* **2007**, *35*, S27–S64.

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.