PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07618, 2025
DOI: 10.1590/1678-5150-PVB-7618

Original Article
Livestock Diseases

Global distribution of antimicrobial resistance in pathogenic *Escherichia coli* isolated from calves: a systematic review[1]

Dircéia A.C. Custódio[2], Amanda C.R. Ferreira[2], Maysa S. Gonçalves[2], Anna C.T.R.B. Costa[2], Carine R. Pereira[2], Fernanda M. Coura[3], Andrey P. Lage[4], Geraldo M. Costa[2], Elaine M.S. Dorneles[2]*

ABSTRACT.- Custódio DAC, Ferreira ACR, Gonçalves MS, Costa ACTBR, Pereira CR, Coura FM, Lage AP, Costa GM, Dorneles EMS. **Global distribution of antimicrobial resistance in pathogenic Escherichia coli isolated from calves: a systematic review.** *Pesquisa Veterinária Brasileira 45:e07618, 2025.* Departamento de Medicina Veterinária, Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras, Campus Universitário, Cx. Postal 3037, Lavras, MG 37200-900, Brazil. E-mail: elaine.dorneles@ufla.br

The present study aimed to perform a systematic review to determine the antimicrobial resistance (AMR) profile of pathogenic *Escherichia coli* strains isolated from the intestinal tract of calves worldwide. Six databases were systematically searched (CABI, Cochrane, PubMed, SciELO, Scopus and Web of Science) with no restrictions regarding the year or place of the publications. A total of 932 studies were recovered, and 56 articles, published from 1982 to 2020, were included in this systematic review. These articles were selected based on title, abstract and full text. The most used technique to determine the susceptibility to antimicrobials of *E. coli* strains was the disk diffusion test (83.93%, 47/56), followed by the minimum inhibitory concentration (MIC) test (19.64%, 11/56). Only two studies (3.57%, 2/56) performed both tests. Seventy-seven different antimicrobial drugs of 17 classes were tested using disk diffusion methodology. For the MIC, fifteen antimicrobial classes and sixty-one antimicrobial drugs were tested. Cephalosporins were the most tested antimicrobial class, both by disk diffusion and MIC methods. Antimicrobial classes with the highest resistance levels were observed for tetracyclines, penicillins, folate inhibitors, aminoglycosides, phenicols and fluoroquinolones. Due to the heterogeneity and low quality of the studies, mainly regarding the antimicrobial susceptibility test methodology used, it was not possible to perform a meta-analysis. The findings showed the global spread of antimicrobial resistance in pathogenic *E. coli* from calves and indicate the importance of carrying out studies based on well-designed analyses to better understand the real emergence and spread of AMR in this pathogen.

INDEX TERMS: Epidemiology, Enterobacteriaceae, cattle, antimicrobial susceptibility, enteropathogenic *Escherichia coli*.

RESUMO.- [**Distribuição global da resistência antimicrobiana em *Escherichia coli* patogênica isolada de bezerros: uma revisão sistemática.**] O presente estudo teve como objetivo realizar uma revisão sistemática para determinar o perfil de resistência antimicrobiana (RAM) de cepas patogênicas de *Escherichia coli* isoladas do trato intestinal de bezerros em todo o mundo. Foram pesquisadas sistematicamente seis bases de dados (CABI, Cochrane, PubMed, SciELO, Scopus e Web of Science) sem restrições quanto ao ano ou local das publicações. Foram recuperados 932 estudos, e 56 artigos, publicados entre 1982 e 2020, foram incluídos nesta revisão sistemática. Esses artigos foram selecionados com base no título, resumo

[1] Received on March 7, 2025.
Accepted for publication on May 9, 2025.
[2] Departamento de Medicina Veterinária, Faculdade de Zootecnia e Medicina Veterinária, Universidade Federal de Lavras (UFLA), Campus Universitário, Cx. Postal 3037, Lavras, MG 37200-900, Brazil. *Corresponding author: elaine.dorneles@ufla.br

[3] Departamento de Ciências Agrárias, Instituto Federal de Minas Gerais (IFMG), Campus Santa Luzia, Rua Veríssimo, 317, Bairro Londrina, Santa Luzia,33115390, Brazil.

[4] Departamento de Medicina Veterinária Preventiva, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Campus Pampulha, Av. Pres. Antônio Carlos 6627, São Francisco, Belo Horizonte, MG 30123970, Brazil.

e texto completo. A técnica mais utilizada para determinar a suscetibilidade aos antimicrobianos de cepas de *E. coli* foi o teste de difusão em disco (83,93%, 47/56), seguido pelo teste de concentração inibitória mínima (CIM) (19,64%, 11/56). Apenas dois estudos (3,57%, 2/56) realizaram ambos os testes. Setenta e sete diferentes antimicrobianos de 17 classes foram testados usando a metodologia de difusão em disco. Para o MIC, foram testadas quinze classes antimicrobianas e sessenta e um fármacos antimicrobianos. As cefalosporinas foram a classe antimicrobiana mais testada, tanto pelos métodos de difusão em disco quanto pelo MIC. As classes antimicrobianas com maiores níveis de resistência foram observadas para tetraciclinas, penicilinas, inibidores de folato, aminoglicosídeos, fenicóis e fluoroquinolonas. Devido à heterogeneidade e baixa qualidade dos estudos, principalmente quanto à metodologia de teste de suscetibilidade antimicrobiana utilizada, não foi possível realizar uma meta-análise. Os achados mostraram a disseminação global da resistência antimicrobiana em *E. coli* patogênica de bezerros e indicam a importância da realização de estudos baseados em análises bem delineadas para melhor compreender a real emergência e disseminação da RAM neste patógeno.

TERMOS DE INDEXAÇÃO: Epidemiologia, Enterobacteriaceae, gado, suscetibilidade antimicrobiana, *Escherichia coli* enteropatogênica.

INTRODUCTION

Pathogenic *Escherichia coli* is responsible for important economic losses in cattle, causing reduced animal weight gain, animal mortality and high drug costs, mainly by producing diarrhea (Kolenda et al. 2015). In this context, some *E. coli* pathotypes are particularly important in the pathogenesis of diarrhea in calves, such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), Shiga toxin-producing *E. coli* (STEC) and necrotoxigenic *E. coli* (NTEC)(Coura et al. 2014). The classification of *E. coli* in pathotypes is based on their attributes of virulence, pathogenesis and clinical signs shown by the host, having different potential to cause disease (Andrade et al. 2012, Cho & Yoon 2014).

In addition to its animal health significance, some *E. coli* pathotypes, such as STEC and EHEC, can also be transmitted through food products to humans, being considered a public health issue (Ray & Singh 2022). Cattle are the main reservoirs for these pathotypes, since they can shed the pathogen in their feces, leading to contamination of the environment, food and water (Coura et al. 2014). Data from the Centers for Disease Control and Prevention (CDC 2021) showed a significant number of 6,034 infections by STEC, including 2,363 infections by STEC O157 *E. coli* strain, evidencing the alarming public health concern that this pathotype represents. Furthermore, some patients with STEC/EHEC infection develop the hemolytic uremic syndrome (HUS), a serious complication mainly associated with serotype O157, characterized by renal failure, hemolytic anemia and thrombocytopenia that can be fatal (Buchanan & Doyle 1997, Joseph et al. 2020).

Another important human health concern associated with *E. coli* infections from animal origin is the worrisome and increasing antimicrobial resistance (AMR), being one of the greatest challenges of the 21st century (Aslam et al. 2021) dissemination of AMR, including multidrug resistance (MDR), is a global problem and a One Health priority, as new forms of resistance can emerge and spread rapidly across continents through people, animals, and environments (Aslam et al. 2021). In addition, according to the World Health Organization (WHO), most antimicrobial drugs are prescribed inappropriately, and most countries do not implement basic policies to promote the rational use of medicines in animal and human health (WHO 2021). Therefore, it is important to perform studies to monitor the trends, distribution and patterns of AMR emergence and dissemination in *E. coli* strains.

The present study aimed to carry out a systematic review to assess the AMR among pathogenic *E. coli* isolated from the intestinal tract of calves worldwide, in order to support decisions on public policies for animal and human health and to diagnose the current scenario of drug resistance in this important pathogen.

MATERIALS AND METHODS

Ethical approval. There are no ethical implications related to the manuscript, since it is a review article, without direct involvement with human beings or animals.

In the present review, the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were adopted (Table S1) (Page et al. 2021).

Search strategy. The systematic search was performed on May 6, 2020, using the following databases: CABI, Cochrane, PubMed, SciELO, Scopus and Web of Science. It was carried out based on the following keywords searched within title, abstract and full text: (bovine* OR cattle OR calves OR calf OR heifer* OR cow* OR herd* OR farm*) AND (pathogenic* OR pathotype* OR virulence gene* OR virulence factors OR virulence*) AND (*Escherichia coli*) AND (antimicrobial OR antibiotic OR resistant* OR susceptibility OR minimal inhibitory concentration OR MIC OR disk diffusion OR resistance gene* OR antimicrobial resistance gene* OR drug resistant*) AND (intestinal tract OR diarrhea), without restrictions regarding the year of publication or place where the study was performed. Details of the search terms used are described in Table S2.

The records retrieved were imported into EndNote X7.8 (Thomson Reuters, USA), and duplicates were removed.

Selection criteria. In the initial stage of selection, the studies were selected based on their titles by two reviewers (DACC and ACRP). Right after, the two reviewers (DACC and ACRP) independently evaluated each abstract. Then, the full text of the articles selected based on their abstract was screened considering the inclusion/exclusion criteria. At any stage, when the two reviewers disagreed, a third reviewer (EMSD) was responsible for the final decision.

Inclusion and exclusion criteria. The selection of studies focused on the assessment of antimicrobial susceptibility through *in vitro* tests of pathogenic *E. coli* isolated from the intestinal tract or feces of calves. Articles written in languages other than English, Spanish, French or Portuguese, as well as those for which full text was not available or that were no original research papers (proceedings, thesis, abstract, book chapter and reviews) were excluded. Full inclusion and exclusion criteria were described in Table S3.

Quality assessment. The evaluation of the quality of the papers included by eligibility was carried out by two authors (DACC and EMSD) based on the following criteria: (i) antimicrobial susceptibility test used (disk diffusion or minimal inhibitory concentration – MIC); (ii) the use of reference standards for performance and interpretation of antimicrobial susceptibility tests (Kahlmeter et al. 2006); (iii) information on the concentration of tested antimicrobials (disk

concentration or MIC range); (vi) information on the breakpoint or halo diameter for the classification of the strains as resistant or susceptible; (v) use of quality control strains in the assays. All criteria were evaluated qualitatively and quantitatively, with the same weight in all selected papers.

Data extraction. Data was extracted by one of the reviewers (DACC) and then checked for accuracy by another reviewer (EMSD). Extracted data from the included studies were as follows: first author, geographic location of the study, year of bacterial isolation, target population, type of study, type of livestock production (when available), type of clinical sample (when available), number of clinical samples (when available), number of animals (when available), age of animals (when available), number/frequency of positive animals (when available), frequency of diarrhea (when available), number of bacterial isolates, number of pathogenic isolates, diagnostic method used (culture and isolation, biochemical test, PCR), genotypes of resistance (when available), antimicrobial susceptibility test(s) used (method, standard reference, quality control, antimicrobial concentration, etc.) and pathogenicity assessment method used (phenotypic or genotypic).

Statistical analysis. Data extracted from the included papers were imported into R statistical software version 4.2.1 (R-4.5.1 for Windows 2022), and a descriptive analysis was performed. The figures were built using the packages ggplot2 (Wickham 2016), plotly (Sievert et al. 2017) and circlize (Gu et al. 2014). Numerical variables were analyzed by calculating the mean, standard deviation, median and interquartile range (IQR), whereas categorical variables were shown as frequency distributions.

RESULTS

General characteristics of the studies included

The initial search identified 932 articles that were assessed for eligibility. After removing 199 duplicates, 733 were screened by title and abstract, according to inclusion/exclusion criteria. Of these, 619 articles were excluded, leaving 114 articles, from which 13 records were not retrieved. Therefore, a total of 101 articles were screened by full text, from which 45 were excluded, leaving 56 articles eligible for inclusion in the systematic review and subject to evaluation of quality criteria (Fig. 1, Table S4 and S5).

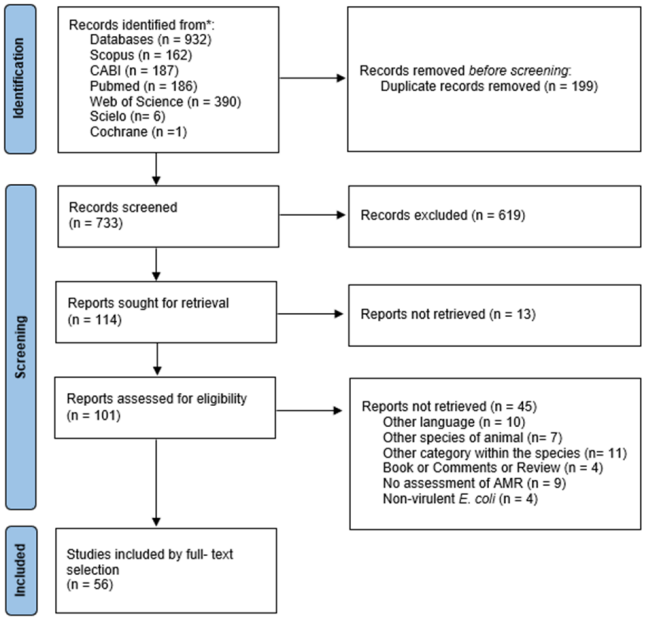

Fig. 1. PRISMA flow diagram of selected studies.

The temporal and geographical distribution of the articles selected in the present study is shown in Figure 2-3. Most of the papers included in the review were published in 2019 (17.85%, 10/56), followed by 8.97% (5/56) published in 2017, 7.14% (4/56) in 2006, 2012, 2014 and 2015 each, 5.35% (3/56) in 2011, and 3.57% (2/56) in 2005, 2008 and 2018 each. Only one study (1.78%) on antimicrobial-resistant *Escherichia coli* from calves was selected from the years 1982, 1988, 1989, 1996, 1999, 2000, 2001, 2002, 2004, 2010, 2013, 2016, and 2020.

Regarding the geographical distribution of the papers, studies were mainly published in India (16.07%, 9/56), followed by Egypt (10.71%, 6/56), USA and Spain (8.92%, 5/56 each), Brazil (7.14%, 4/56), China, France, Italy and South Africa (5.35%, 3/56 each), and Argentina, Iran and Turkey (3.57%, 2/56 each). Countries with only one study published were (1.78%, 1/56) Bangladesh, Belgium, Canada, Chile, Pakistan, Sweden, Tanzania and Uruguay. One study (1.78%, 1/56) did not inform where it was carried out (Khalifa et al. 2019) (Fig. 2).

Among the selected papers, 83.92% (47/56) reported isolation of pathogenic *E. coli* strains from the intestinal tract of calves and 16.08% (9/56) from buffalo calves. Concerning the sampling collection, 78.57% (44/56) performed *E. coli* isolation from stool samples, 14.28% (8/56) from rectal swabs and 1.78% (1/56) from intestinal content. In contrast, three studies (5.36%) did not report which clinical sample was used, although they stated that the strains were isolated from diarrheic calves. Regarding the type of livestock production, 41.07% (23/56) of the studies were conducted in dairy farms, 3.35% (3/56) in dairy/beef farms, and 53.57% (30/56) did not inform the type of cattle production. Most of the papers adopted the cross-sectional study design (58.92%, 33/56), while 5.35% (3/56) were case-control studies and 35.71% (20/58) did not follow any study design.

The frequency of diarrhea reported in the studies ranged from 0 to 100%, and 14.28% (8/56) of the studies had no information on diarrhea occurrence. The number of clinical samples tested per study ranged from 4 to 824, with a mean of 212.55 (± 199.94), a median of 118.50 (IQR 247), and 14 articles did not report the number of tested samples.

The number of animals sampled varied from 16 to 600, with a mean of 165.19 (± 157.31) and a median of 107.5 (IQR 114.14). This information was not available in 42.86% (24/56) of articles. The number of *E. coli* isolates ranged from 1 to 700, with mean of 133.04 (± 152.25) and median of 87 (IQR 175.25), and the number of isolates considered pathogenic (positive for at least one virulence factor tested in the study by phenotypic or genotypic methods) ranged from 1 to 419, with mean of 45.10 (± 66.43) and median of 18 (IQR 55). The isolates were confirmed as *E. coli* by a species-specific polymerase chain reaction (PCR) only in 26.78% (15/56) of the studies, while the others used biochemical tests for species identification.

The main characteristics of the selected studies are summarized in Figure S1 and will be further detailed in the following sections.

Characterization of virulence in *E. coli* strains

Escherichia coli virulence was evaluated by PCR of virulence genes in 82.14% (46/56) of the studies (Table 1), whereas 23.21% (13/56) assessed the pathogenicity of the strains by different phenotypic assays (Table 2). The virulence genes assessed and the frequency of studies in which they were observed are shown in Table 1.

The main virulence genes investigated in the articles were: Shiga toxins (*stx, stx1, stx2, stx2a, stxb, stxc, stxd, stx2e, stx2g, vt, vt2e, vtx*, and *vtx2*) (76.08%, 35/46), intimin (*eae* and *eaeA*) (60.86%, 28/46), fimbrial adhesins (*F4, F5, F6, F17, F17c, F17g, F17f, F18* and *F41*) (26.09%, 12/46), thermolabile enterotoxins (*IntII_LT*, *elt*, and *eltA*) (23.91%, 11/46), hemolysins (*hlyA* and *hlyF*) (21.74%, 10/46), thermostable enterotoxins (*st, sta*, and *stb*) (21.74%, 10/46), enterohemolysin (*ehxA, ehlyA*, and *ehly*) (17.39%, 8/46), necrotizing factor (*cnf1* and *cnf2*) (15.22%, 7/46), cytolethal distending toxins (*cdt, cdtb,* and *cdtIII*) (8.70%, 4/46).

Regarding serotyping, 64.29% (36/56) of the studies determined serogroups, of which 77.78% (28/36) performed serotyping by serum agglutination test, 13.89% (5/36) identified serogroups by PCR, 5.55% (2/36) by genetic serotyping (whole genome sequencing) and 2.78% (1/36) did not inform the methodology used.

Characteristics of the antimicrobial susceptibility tests

The most used technique to determine antimicrobial susceptibility was the disk diffusion test (83.93%, 47/56), followed by MIC (19.64%, 11/56). Only two studies (3.57%, 2/56) performed both tests (disk diffusion and MIC) (Fig. 3). The E-test was not used by any of the selected studies.

Regarding the procedures, 57.14% (32/56) of the studies followed the methodology and interpretation parameters

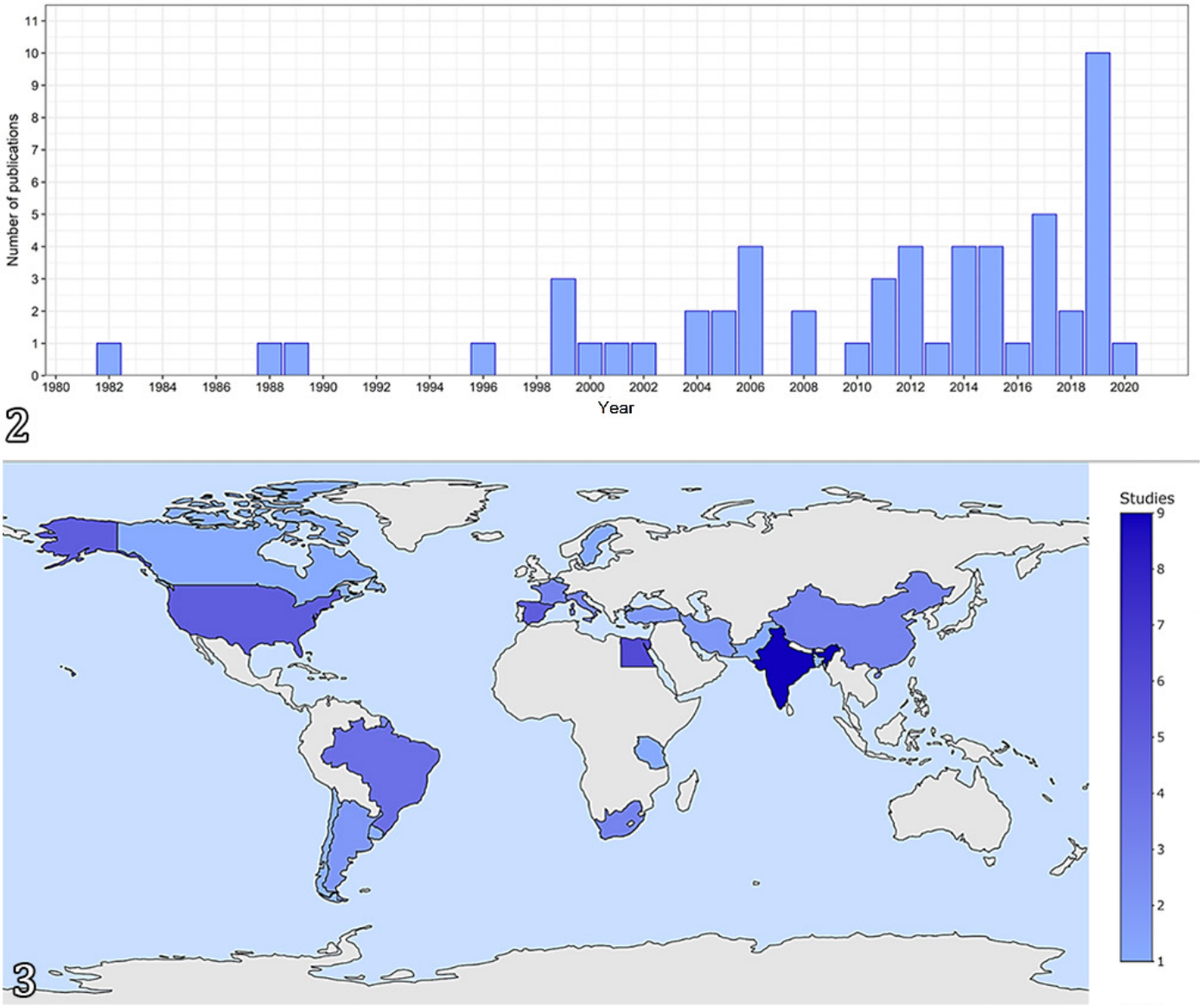

Fig. 2-3. Temporal and geographical distribution of the selected articles. (**2**) Distribution of the articles included by eligibility according to the year of publication. (**3**) Distribution of the articles included by eligibility according to the country where the study was performed.

Table 1. Frequency of prospection and identification of virulence mechanisms in studies selected by this systematic review on *Escherichia coli* isolated from the intestinal tract of calves and buffalo calves, published from 1982 to 2020

Gene/target	Virulence mechanism	N of studies that tested (%)	Identified
afa	Afimbrial adhesin	1/46 (2.17)	Yes
air	Autotransporter adhesin	1/46 (2.17)	Yes
aggR	Adherence transcriptional regulator	1/46 (2.17)	Yes
astA	Thermostable cytotonic enterotoxin	5/46 (10.87)	Yes
bcsA	Bacterial cellulose synthesis	1/46 (2.17)	Yes
bfpA	Main subunit of the *bfp* fimbria, which enables bacterial aggregation	6/46 (13.04)	Yes
cma	Encodes bacteriocins/microcins	2/46 (4.35)	Yes
cnf	Cytotoxic necrotizing factor	2/46 (4.35)	Yes
cnf1	Cytotoxic necrotizing factor	5/46 (10.87)	Yes
cnf2	Cytotoxic necrotizing factor	6/46 (13.04)	Yes
cia	Protein secretion (invasion antigen)	1/46 (2.17)	Yes
clp-g	Protein secretion (type VI secretion system)	1/46 (2.17)	Yes
cs31A	Capsule-like antigen	1/46 (2.17)	Yes
cvaA	secretion protein (colicin V)	1/46 (2.17)	Yes
crl	Fimbria curli regulator	1/46 (2.17)	Yes
csgA	Temperature regulated curli filament	2/46 (4.35)	Yes
csgD	Temperature regulated curli filament	1/46 (2.17)	Yes
Ccdt	Cytolethal distending toxins	1/46 (2.17)	Yes
cdtB	Cytolethal distending toxins	1/46 (2.17)	NI
cdtIII	Cytolethal distending toxins	2/46 (4.35)	Yes
eaeA	Adhesion factor plasmid	1/46 (2.17)	Yes
etsC	Response regulator	1/46 (2.17)	Yes
eitB	Virulence transcriptional regulator	1/46 (2.17)	Yes
eilA	Virulence transcriptional regulator	1/46 (2.17)	Yes
east1	Aggregative adherence fimbriae	1/46 (2.17)	Yes
est	Carboxyl hydrolases/esterase	3/46 (6.52)	Yes
estA	Carboxyl hydrolases/esterase	2/46 (4.35)	Yes
let	Heat-labile enterotoxin	3/46 (6.52)	Yes
eltA	Heat-labile enterotoxin	1/46 (2.17)	Yes
efa	Factor for adherence	1/46 (2.17)	Yes
espA	Type III secretion system translocator protein	1/46 (2.17)	Yes
espB	Type III secretion system translocator protein	1/46 (2.17)	Yes
espF	Type III secretion system effector E	1/46 (2.17)	Yes
espP	Serine protease. cleaves coagulation factor V	2/46 (4.35)	Yes
ehxA	Enterohemolysin	3/46 (6.52)	Yes
ehly	Enterohemolysin	2/46 (4.35)	Yes
ehlyA	Enterohemolysin	1/46 (2.17)	Yes
eae	Intimin-like adhesin	27/46 (58.70)	Yes
eaeA	Intimin-like adhesin	5/46 (10.87)	Yes
etpD	Type II secretion system secretin	1/46 (2.17)	Yes
ehaAα	Autotransporter adhesin	1/46 (2.17)	Yes
ehaAβ	Autotransporter adhesin	1/46 (2.17)	Yes
escv	Secretion system genes	2/46 (4.35)	Yes
fyuA	Siderophore receptor	1/46 (2.17)	Yes
F4 (K88)	Fimbrial adhesive	3/46 (6.52)	Yes
F5 (K99)	Fimbrial adhesive	12/46 (26.09)	Yes
F6(987P)	Fimbrial adhesive	3/46 (6.52)	Yes
F17	Fimbrial adhesive	1/46 (2.17)	Yes
F17c	Fimbrial adhesive	2/46 (4.35)	Yes
F17g	Fimbrial adhesive	1/46 (2.17)	Yes
F18	Fimbrial adhesive	4/46 (8.70)	Yes
F41	Fimbrial adhesive	9/46 (19.56)	Yes
fimH	Protein precursor	3/46 (6.52)	Yes

Gene/target	Virulence mechanism	N of studies that tested (%)	Identified
flu	Cell self-aggregation	1/46 (2.17)	Yes
fiCH4	Flagellin	1/46 (2.17)	Yes
gad	Acid resistance	2/46 (4.35)	Yes
hlyA	Hemolysin	8/46 (14.29)	Yes
hglyF	Hemolysin	2/46 (4.35)	Yes
ibeA	Invasion protein	1/46 (2.17)	Yes
Iha	Siderophore receptor/adhesin	2/46 (4.35)	Yes
ireA	Siderophore receptor	2/46 (4.35)	Yes
iroN	Iron acquisition	2/46 (4.35)	Yes
irp-2	Polyketide synthase	1/46 (2.17)	Yes
Iss	Serum survival gene	3/46 (6.52)	Yes
lucC	Siderophore biosynthesis protein	1/46 (2.17)	Yes
lucD	L-lysine 6-monooxygenase	1/46 (2.17)	Yes
lpfA	Long polar fimbriae	1/46 (2.17)	Yes
intII_LT	Heat-labile enterotoxins	8/46 (14.29)	Yes
katP	Catalase/peroxidase	1/46 (2.17)	Yes
kpsMII	Polysialic acid transport protein	1/46 (2.17)	Yes
malx	Encodes enzyme II of the phosphotransferase system	1/46 (2.17)	Yes
mchF	Microcin transport protein	1/46 (2.17)	Yes
mcmA	Protein microcin-bacteriocin	1/46 (2.17)	Yes
mchB	Protein microcin-bacteriocin	1/46 (2.17)	Yes
mchC	Protein microcin-bacteriocin	1/46 (2.17)	Yes
mchF	Protein microcin-bacteriocin	1/46 (2.17)	Yes
mleA	Type III secretion system effector	1/46 (2.17)	Yes
nleB	Type III secretion system effector	1/46 (2.17)	Yes
nleC	Type III secretion system effector	1/46 (2.17)	Yes
ompA	Membrane protein (iron resistance)	1/46 (2.17)	Yes
ompTp	Membrane protein(proteases)	1/46 (2.17)	Yes
plnv	Integral membrane protein	1/46 (2.17)	Yes
papC	Protein fimbrial	1/46 (2.17)	Yes
papG	Adhesin fimbrial	1/46 (2.17)	Yes
papG (Allelle I)	P-Fimbrial outer membrane protein	1/46 (2.17)	Yes
papG (Allelle II	P-Fimbrial outer membrane protein	1/46 (2.17)	Yes
papG (Allele III)	P-Fimbrial outer membrane protein	1/46 (2.17)	Yes
papAH	P-Fimbrial outer membrane protein	1/46 (2.17)	Yes
rbfp O157/fliCH7	Somatic and flagellar antigen	1/46 (2.17)	Yes
rpoS	Regulator sigma factor	1/46 (2.17)	Yes
sitA	Iron/manganese ABC transporter substrate-binding protein	1/46 (2.17)	Yes
st	Thermostable enterotoxins	1/46 (2.17)	Yes
sta	Heat-stable enterotoxin	10/46 (2.17)	Yes
stb	Heat-stable enterotoxin	4/46 (8.70)	Yes
stx	Shiga toxin	2/46 (4.35)	Yes
stx1	Shiga toxin	31/46 (67.39)	Yes
stx2	Shiga toxin	26/46 (56.52)	Yes
stx1/stx2	Shiga toxin	1/46 (2.17)	Yes
stx2a	Shiga toxin	1/46 (2.17)	NI
stx2b	Shiga toxin	1/46 (2.17)	NI
stx2c	Shiga toxin	3/46 (6.52)	Yes
stx2d	Shiga toxin	1/46 (2.17)	NI
stx2e	Shiga toxin	3/46 (6.52)	Yes
stx2f	Shiga toxin	1/46 (2.17)	NI
stx2g	Shiga toxin	1/46 (2.17)	NI
sfa/focDE	Gene related to biofilm formation	1/46 (2.17)	Yes
tccp	Cytoskeleton coupling protein	1/46 (2.17)	Yes
tsh	Temperature sensitive hemagglutinin	1/46 (2.17)	Yes

Gene/target	Virulence mechanism	N of studies that tested (%)	Identified
tir	Intimin receptor Tir	1/46 (2.17)	Yes
traT	Lipoprotein	1/46 (2.17)	Yes
toxB	Cytotoxin	1/46 (2.17)	Yes
vt	Vero cytotoxin (verotoxicin in vero cells)	1/46 (2.17)	NI
vt2e	Vero cytotoxin (verotoxicin in vero cells)	1/46 (2.17)	NI
vtx	Vero cytotoxin (verotoxicin in vero cells)	1/46 (2.17)	Yes
vtx2	Vero cytotoxin (verotoxicin in vero cells)	1/46 (2.17)	Yes
wzxo	Putative O-antigen flippase	1/46 (2.17)	No
uidA	Encodes the beta-glucuronidase enzyme	2/46 (4.35)	Yes

NI = not informed.

Table 2. Frequency of studies that performed virulence phenotypic assays in studies selected on *Escherichia coli* isolated from the intestinal tract or feces of calves, published from 1982 to 2020

Target	Method (N of studies)	Total N of studies that tested (%)	Identified
Adhesion assay	Inoculation of Hep-2 cells, tissue culture plates	2/13 (15.38)	Yes
Biofilm	*In vitro* biofilm induction by the polystyrene microtiter method by the Cristal Violet method	1/13 (7.69)	NI
Cytotoxic necrotizing factor	NI	1/13 (7.69)	Yes
Fimbrial adhesive *F4 (K88)*	NI	1/13 (7.69)	Yes
Fimbrial adhesive *F5 (K99)*	Agglutination on plates with antiserum (2)/NI (3)	5/13 (38.46)	Yes
Fimbrial adhesive *F17*	NI	2/13 (15.38)	Yes
Fimbrial adhesive *F41*	NI	2/13 (15.38)	Yes
Fimbrial adhesive *K101*	NI	2/13 (15.38)	Yes
Enterohemolysin	Detection in sheep blood plates	1/13 (7.69)	Yes
Hemolysin	Detection in sheep blood plates	1/13 (7.69)	Yes
α-hemolysin	Detection in sheep blood plates	1/13 (7.69)	No
Heat-labile enterotoxins	*In vitro* inoculation of vero cells (2)/NI (2)	4/13 (30.76)	NI
Shiga-toxin	*In vitro* inoculation of vero cells	2/13 (15.38)	Yes

NI = not informed.

described by Clinical & Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (Eucast) and 23.21 % (13/56) followed the parameters proposed by (Bauer et al. 1966), whereas 12.58% (7/56) followed other references and four studies (7.14%, 4/56) did not inform the adopted protocol. Most studies did not present quality control (QC) strains (69.64%, 39/56), which was informed only by 30.36% (17/56) of the studies. Regarding the concentration of antimicrobials used in the tests, most studies reported the concentration used (64.29%, 36/56) and 35.71% (20/56) did not provide this information (Table S6).

Antimicrobial classes and drugs used for the disk diffusion tests

Among the studies included in this systematic review, 47 (83.93%) used the disk diffusion method to assess antimicrobial susceptibility of *E. coli* strains. The percentages of studies that tested and observed resistance to different antimicrobial classes and drugs, as well as the concentrations used, are shown in Table 3.

Seventeen antimicrobial classes and 78 different antimicrobial drugs were used in the disk diffusion tests. The most tested antimicrobial class was cephalosporin, among which 16 different antimicrobials drugs were tested (20.51%, 16/78), followed by penicillins (19.23%, 15/78), fluoroquinolones (12.82%, 10/78), aminoglycosides and folate inhibitors (10.26%, 8/78 each), macrolides, tetracyclines and lincosamides (3.85%, 3/78 each), carbapenems, phenicols, and polymyxins (2.56%, 2/78 each), and aminocoumarins, fosfomycins, quinolones, macrocyclic, monobactam, and nitrofurans (1.28%, 1/78 each).

Among cephalosporins, the most tested antimicrobial within the class was cefotaxime, being reported in 21.27% of the studies (10/47), followed by cephalothin (19.15%, 9/15); ceftazidime (17.02%, 8/47); ceftiofur (14.89%, 7/47); cefepime (12.77%, 6/47); cefuroxime and ceftriaxone (10.64%, 5/47 each); cefalexin (6.38%, 3/47); ceftriaxone, cefoperazone and cefoxitin (4.25%, 2/47 each); cephaloridine, cefazolin, cefixime, cefaclor, and cephalonium (2.13%, 1/47 each).

Regarding penicillins, ampicillin was the main antimicrobial tested (65.96%, 31/47), followed by amoxicillin/clavulanic acid (25.53%, 12/47); amoxicillin (23.40%, 12/46); penicillin G (12.77%, 6/47), cloxacillin, and piperacilline/tazobactam (4.26%, 2/47 each), and amdinocillin, amoxiclav, ampicillin/sulbactam, mezlocillin, oxacillin, tazobactam, temocillin, ticarcillin, and ticarcillin/clavulanic acid (2.13%, 1/47 each).

Enrofloxacin was the main antimicrobial tested among the fluoroquinolones, present in 44.68% (21/47) of the studies, followed by ciprofloxacin (38.30%, 18/47); norfloxacin (27.66%, 13/47); and marbofloxacin and levofloxacin (4.25%, 2/47 each). In addition to enrofloxacin, six other fluoroquinolones were tested, all of which were present in only one study: danofloxacin, flumequine, ofloxacin, and pefloxacin (2.13%, 1/47 each).

For the aminoglycosides class, gentamicin was the most frequent with 72.34% (34/47), followed by streptomycin

Table 3. Number of studies that tested and observed resistance to several antimicrobials using the disk-diffusion technique on *Escherichia coli* isolated from the intestinal tract or feces of calves, published from 1982 to 2020

Class	Antimicrobial	Tested concentration (N of studies)	Total N of studies that tested (%)	N of studies that observed resistance (%)
Aminocoumarin	Novobiocin	NI	1/47 (2.13)	NI/1
Aminoglycosides	Amikacin	30 µg (9) / 30 mg (1) / NI (4)	14/47 (29.79)	5/14 (35.71)
	Apramycin	15 µg (2)	2/47 (4.25)	2/2 (100)
	Gentamicin	10 µg (21) / 30 µg (2) / 10 mg (3) / NI (8)	34/47 (72.34)	18/34 (52.94)
	Kanamycin	10 µg (2) / 30 µg (9) / 30 µg / NI (7)	18/47 (38.30)	12/18 (66.66)
	Neomycin	20 µg (1) / 25 µg (1) / 30 µg (9) / 30mg (1) / NI (2)	14/47 (29.79)	7/14 (50.0)
	Spectinomycin	10 µg (1) / 20 µg (1) / 100 µg (2)	4/47 (8.51)	4/4 (100)
	Streptomycin	10 µg (11) / 10 mg (3) / NI (9)	23/47 (48.94)	12/23 (52.17)
	Tobramycin	10 µg (1)	1/47 (2.13)	1/1 (100)
Carbapenems	Imipenem	10 µg (2) / NI (3)	5/47 (10.64)	0/5 (0.00)
	Meropenem	10 µg (1) / NI (4)	5/47 (10.64)	2/5(40.0)
Cephalosporins	Cefaloridine	10 µg (1)	1/47 (2.13)	1/1 (100)
	Cefazolin	30 µg (1)	1/47 (2.13)	1/1 (100)
	Cefepime	30 µg (4) / NI (2)	6/47 (12.77)	1/6 (16.66)
	Ceftrizone	30 mg (1) / NI (1)	2/47 (4.25)	NI/2
	Cefixime	5 µg (1) / NI (1)	1/47 (2.13)	1/1 (100)
	Cefoperazone	75 mg (1) / NI (1)	2/47 (4.25)	NI/2
	Cefotaxime	30 µg (6) / NI (4)	10/47 (21.27)	3/10 (30.00)
	Cefoxitin	30 µg (2)	2/47 (4.26)	0/2 (0.00)
	Ceftazidime	30 µg (4) / NI (4)	8/47 (17.02)	4/8 (50.00)
	Ceftiofur	0.2 µg (1) / 30 µg (6)	7/47 (14.89)	4/7 (57.14)
	Ceftriaxone	30 µg (2) / NI (3)	5/47 (10.64)	2/5 (40.0)
	Cefuroxime	30 µg (4) / NI (1)	5/47 (10.64)	5/5 (100)
	Cephachlor	NI (1)	1/47 (2.13)	1/1 (100)
	Cephalexin	30 µg (2) / NI (1)	3/47 (6.38)	3/3 (100)
	Cephalonium	NI (1)	1/47 (2.13)	1/1 (100)
	Cephalothin	30 µg (7) / NI (2)	9/47 (19.15)	7/9 (77.78)
Fluoroquinolones	Ciprofloxacin	5 µg (9) / 5 mg (2) / 10 µg (1) / NI (6)	18/47 (38.30)	8/18 (44.44)
	Danofloxacin	NI (1)	1/47 (2.13)	NI/1
	Enrofloxacin	5 µg (11) / 10 µg (5) / 5 mg (1) / 10 mg (1) / NI (3)	21/47 (44.68)	12/21 (57.14)
	Flumequine	30 µg (1)	1/47 (2.13)	1/1 (100)
	Levofloxacin	5 µg (1) / NI (1)	2/47 (4.25)	0/2 (0.00)
	Marbofloxacin	10 µg (2)	2/47 (4.25)	2/2 (100)
	Norfloxacin	5 µg (1) / 10 µg (10) / 10 mg (1) / NI (1)	13/47 (27.66)	8/13 (61.54)
	Ofloxacin	NI (1)	1/47 (2.13)	0/1 (0.00)
	Perfloxacin	5 µg (1)	1/47 (2.13)	1/1 (100)
Folate inhibitor	Cotrimoxazole	25 µg (4) / NI (2)	6/47 (12.77)	4/6 (66.66)
	Sulfadiazine	300 mg (1) / NI (2)	3/47 (6.38)	NI/3
	Sulfamethoxazole	25 µg (1) / NI (4)	5/47 (10.64)	4/5 (80.00)
	Sulfamethoxazole-trimethoprim	25 µg (16) / 25 mg (1) / 30 µg (1) / NI (5)	23/47 (48.94)	16/23 (69.56)
	Sulfaprim	50 µg (1)	1/47 (2.13)	1/1 (100)
	Sulfonamides	30 µg (1) / 300 µg (1) / NI (3)	5/47 (10.64)	2/5 (40.00)
	Trimethoprim	5 µg (2) / 5 mg (1) / NI (3)	7/47 (14.89)	4/7 (57.14)
	Trimethoprim -sulfadiazine	25 µg (1)	1/47 (2.13)	NI/1
Fosfomycins	Fosfomycin	NI (1)	1/47 (2.13)	NI/1
Lincosamides	Clindamycin	2 µg (1)	1/47 (2.13)	NI/1
	Lincomycin	2 µg (2) / NI (2)	4/47 (8.51)	2/4 (50.00)
	Lincospectin	100 µg (1) / NI (1)	2/47 (4.25)	0/2 (0.00)
Macrocyclic	Rifampicin	5 µg (1)/NI (1)	2/47 (4.25)	2/2 (100)

Class	Antimicrobial	Tested concentration (N of studies)	Total N of studies that tested (%)	N of studies that observed resistance (%)
Macrolides	Erythromycin	15 µg (3) / 25 µg (1) / NI (3)	7/47 (14.89)	4/7 (57.14)
	Espiramycin	NI (1)	1/47 (2.13)	1/1 (100)
	Thiomicosin	NI (1)	1/47 (2.13)	1/1 (100)
Monobactam	Aztreonam	30 µg (1) / NI (3)	4/47 (8.51)	2/4 (50.00)
Nitrofurans	Nitrofurantoin	30 µg (1) / 300 µg (2) / NI (3)	6/47 (12.77)	4/6 (66.66)
Penicillin	Amdinocillin	NI (1)	1/47 (2.13)	0/1 (0.00)
	Amoxicillin	10 µg (4) / 25 µg (2) / 30 µg (3) / 20 mg (1) / NI (2)	11/47 (23.40)	9/11(81.82)
	Amoxicillin/ clavulanic acid	10 µg (1) / 30 µg (2) / 10 mg (1) / 20/10 µg (4) / NI (4)	12/47 (25.53)	7/12 (58.33)
	Amoxiclav	NI (1)	1/47 (2.13)	NI/1
	Ampicillin	10 µg (19) / 10 mg (3) / NI (9)	31/47 (65.96)	19/31 (61.29)
	Ampicillin/ sulbactam	10/10 µg (1)	1/47 (2.13)	1/1 (100)
	Cloxacillin	5 µg (1) / NI (1)	2/47 (4.25)	2/2 (100)
	Mezlocillin	75 µg (1)	1/47 (2.13)	1/1 (100)
	Oxacillin	1 µg (1)	1/47 (2.13)	1/1 (100)
	Penicillin G	10 µg (4) / 10 mg (1) / NI (1)	6/47 (/12.77)	4/6 (66.66)
	Piperacilline-Tazobactan	NI (2)	2/47 (4.25)	1/2 (50.00)
	Tazobactam	NI (1)	1/47 (2.13)	NI/1
	Temocillin	NI (1)	1/47 (2.13)	0/1 (0.00)
	Ticarcillin	75 µg (1)	1/47 (2.13)	1/1 (100)
	Ticarcillin-clavulanic acid	75/10 µg (1)	1/47 (2.13)	1/1 (100)
Phenicols	Chloramphenicol	10 µg (1) / 30 µg (12) / 30 mg (3) / NI (7)	23/47 (48.94)	14/23 (60.87)
	Florfenicol	30 µg (5) / NI (1)	6/47 (12.77)	3/6 (50.0)
Polymyxins	Colistin	10 µg (5) / 10 mg (1) / NI (1)	7/47 (14.89)	4/7 (57.14)
	Polymyxin B	300 U (1) / NI (1)	2/47 (4.25)	1/2 (50.00)
Quinolones	Nalidixic Acid	10 µg (1) / 30 µg (9) / 30 mg (1) / NI (7)	18/47 (38.30)	9/18 (50.00)
	Doxycycline	30 µg (1)	1/47 (2.13)	1/1(100)
Tetracyclines	Oxytetracycline	30 µg (5) / NI (1)	6/47 (12.76)	6/6 (100)
	Tetracycline	10 µg (1) / 30 µg (19) / 30 mg (3) / NI (9)	32/47 (68.08)	22/32 (68.75)

NI = not informed.

(48.94%, 23/47); kanamycin (38.30%, 18/47); amikacin and neomycin (29.79%, 14/47); spectinomycin (8.51%, 4/47); apramycin (4.25%, 2/47), and tobramycin (2.13%, 1/47). Sulfamethoxazole/trimethoprim was the more frequent among folate inhibitors (48.94%, 23/47), followed by trimethoprim (14.89%, 7/47); cotrimoxazole (12.77%, 6/47), sulfamethoxazole and sulfonamides (10.64%, 5/47 each); sulfadiazine (6.38%, 3/47); sulfaprim and trimethoprim/ sulfadiazine (2.13%, 1/47 each).

Among the macrolides class, erythromycin was the most tested drug (14.89%, 7/47), while espiramycin and tilmicosin were observed in just one study each (2.13%, 1/47). Regarding lincosamides, lincomycin was tested in two studies (8.51%, 4/47), followed by lincospectin (4.25%, 2/47) and clindamycin (2.13%, 1/47). Tetracycline was the main antimicrobial tested among tetracyclines, present in 68.08% (32/47) of the studies, followed by oxytetracycline in 12.76% (6/47), and doxycycline in only one (2.13%, 1/47). Carbapenems were represented by imipenem and meropenem (10.64%, 5/47 each).

Within the phenicol class, chloramphenicol was tested in 48.94% (23/47) of the studies, while florfenicol was tested in 12.77% (6/47). Representing the polymyxin class, colistin was tested in 14.89% (7/47) of the studies, while polymyxin B was tested in two (4.26%, 2/47).

Finally, the following classes were represented by only one antimicrobial each: aminocoumarin, with novobiocin tested in one study (2.13%, 1/47); fosfomycin, with fosfomycin also in one study (2.17%, 1/46); macrocyclic, represented by rifampicin in two studies (4.25%, 2/47); monobactam, with aztreonam, in four studies (8.51%, 4/47); nitrofurans, represented by nitrofurantoin in 12.77% (6/47) of the studies; and quinolones, with nalidixic acid tested in eighteen studies (38.30%, 18/47).

Antimicrobial classes and drugs used for the minimal inhibitory concentration (MIC)

Only 19.64% (11/56) of the studies used MIC to assess antimicrobial susceptibility of *E. coli* strains. Table 4 shows the studies that tested each class and antimicrobial drugs, as well as the concentration ranges and breakpoints adopted.

Fifteen antimicrobial classes were tested, representing a total of 61 drugs. Cephalosporins was the class with more representatives (16.39%, 10/61), followed by penicillins (14.75%, 9/61), aminoglycosides (13.11%, 8/61), fluoroquinolones and folate inhibitors (9.84%, 6/61), macrolides (8.20%, 5/61),

Table 4. Number of studies that tested and observed resistance to several antimicrobials using the microdilution technique on *Escherichia coli* isolated from the intestinal tract or feces of calves, published from 1982 to 2020

Class	Antimicrobial	MIC range µg/mL (N of studies)	Cut-off (N of studies)	Total N of studies that tested (%)	N of studies that observed resistance
Aminoglycosides	Apramycin	1 > 512 (1)	≥ 64 (1)	1/11 (9.09)	NI/1
	Amikacin	2 > 64 (1)	NI (1)	1/11 (9.09)	NI/1
	Gentamicin	0.25-256 (1) / 0.25-32 (1) / 125-512 (1) / 1-16 (1) / 0.5-64 (1) / NI (3)	≥ 2 (2) / ≥ 16 (4) / NI (2)	8/11 (72.72)	5/8 (62.5)
	Kanamycin	0.25-256 (1) / 4-128 (1) / 0.5-512 (1)	≥ 8 (1) / ≥ 64 (2)	3/11 (27.27)	1/3 (33.33)
	Neomycin	0.25-512 (1) / 2-16 (1) / NI (1)	≥ 8 (1) / ≥ 64 (1) / N1 (1)	3/11 (27.27)	1/3 (33.33)
	Streptomycin	0.25-256 (1) / 0.5-512 (1) / 2-256 (1) / 2-128 (1) / NI (1)	≥ 64 (3) / ≥ 16 (1) / > 16 (1)	5/11 (45.45)	3/5 (60.0)
	Spectinomycin	0.5-512 (1) / NI (1)	≥ 64 (1) / N1 (1)	2/11 (18.18)	2/2 (100)
	Tobramycin	1-16 (1)	NI (1)	1/11 (9.09)	0/1 (0.00)
Cephalosporins	Cephazolin	4-64 (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Cephalothin	1-512 (1)	≥ 32 (1)	1/11 (9.09)	NI/1
	Cefuroxime	0.125-64 (1)	≥ 32 (1)	1/11 (9.09))	NI/1
	Cefotaxime	0.06-4 (1) / 0.625-2 (1)	≥ 0.25 (1) / ≥ 64 (1)	2/11 (18.18)	1/2 (50.00) / 1/2 (NI)
	Cefquinome	0.0625-2 (1)	NI (1)	1/11 (9.09)	NI/1
	Cefepime	1-64 (1)	NI (1)	1/11(9.09)	1/1 (100)
	Cefoxitin	0.25-256 (1) / NI (1)	≥ 32 (1) / NI (1)	2/11 (18.18)	1/2 (50.00)
	Ceftazidime	0.25-16 (1) / NI (1)	≥ 0.5 (1) / NI (1)	2/11 (18.18)	1/2 (50.00) / 1/2 (NI)
	Ceftiofur	0.12-16 (1) / 0.25-256 (1) / 16-256 (1) / NI (2)	≥ 8 (2) / ≥ 1(1) / NI (2)	5/11 (45.45)	3/5 (60.00)
	Ceftriaxone	0.25-0.256 (1) / 1-64 (1)	≥ 4 (1) / NI (1)	2/11(18.18)	2/2 (100)
Carbapenems	Imipenem	0.25-16 (1) / NI (1)	NI (2)	2/11(18.18)	1/2 (50.00)
	Ertapenem	0.5-8 (1)	NI (1)	1/11 (9.09)	0/1 (0.00)
	Meropenem	0.25-16 (1)	NI (1)	1/11 (9.09)	1/1 (100)
Phenicol	Florfenicol	0.5-128 (1) / 2-64 (1) / 4-32 (1) / 8-256 (1) / NI (2)	≥ 8 (2) / ≥ 16 (2) / ≥ 32 (1) / NI (1)	6/11 (54.54)	4/6 (66.66)
	Chloramphenicol	0-25-256 (1) / 1-128 (1) / 1-512 (1) / 2-64 (1) / NI (2)	≥ 16 (2) / ≥ 32 (4)	6/11 (54.54)	3/6 (50.00)
Fluorquinolones	Ciprofloxacin	0.008-8 (1) / 0.25-256 (1) / 0.25-4 (1)	≥ 0.06 (1) / ≥ 4 (1) / NI (1)	3/11 (27.27)	1/3 (33.33)
	Enrofloxacin	0.03-4 (1) / 0.0625-64 (1)	> 0.12 (1) / ≥ 2 (1)	2/11(18.18)	NI/2
	Enoxacin	0.0625-256 (1)	≥ 8 (1)	1/11 (9.09)	NI/1
	Danofloxacin	0.0625-256 (1) / NI (1)	NI (2)	2/11 (18.18)	1/2 (50.00) / 1/2 (NI)
	Moxifloxacin	0.25-8 (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Oxolinic acid	0.0625-512 (1)	NI (1)	1/11 (9.09)	NI/1
Lincosamides	Clindamycin	NI (1)	NI (1)	1/11 (9.09)	1/1 (100)
Monobactam	Aztreonam	1-64 (1) / NI (1)	NI (2)	2/11 (18.18)	1/2 (50.00)
Penicillins	Co-amoxiclav	NI (1)	NI (1)	1/11 (9.09)	NI/1
	Amoxicillin/ clavulanic acid	0.25-256 (1)	≥32	1/11 (9.09)	1/1 (100)
	Ampicillin	0.25-256 (1) / 0.5-32 (1). 0.25-32 (1) 1-512 (1) / 128-512 (1) / NI (4) / 2-32 (1)	≥ 32 (3) / > 8 (2) / NI (5)	10/11(90.90)	7/10(70.00)
	Ampicillin/ sulbactam	2/1 – 32/16 (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Penicillin	NI (1)	NI (1)	1/11(9.09)	1/1 (100)
	Piperacillin	NI (1)	NI (1)	1/11 (9.09)	NI/1
	Piperacillin-tazobactam	NI (1)	NI (1)	1/11 (9.09)	NI/1
	Ticarcillin	128-1024 (1) / NI (1)	128 (1) / NI (1)	2/11 (18.18)	1/2 (50.00)
	Ticarcillin/ clavulanic acid	NI (1)	NI (1)	1/11(9.09)	NI/1

Class	Antimicrobial	MIC range µg/mL (N of studies)	Cut-off (N of studies)	Total N of studies that tested (%)	N of studies that observed resistance
Quinolones	Nalidixic acid	0.25-256 / (1) / 0.5-512 (1) / 1-128 (1) / 4-64 (1)	≥ 16 (2) / ≥ 32 (2)	4/11 (36.36)	2/4 (50.00)
Macrolides	Tilmicosin	NI (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Tylosin	8-512 (1)	≥ 8	1/11 (9.09)	NI/1
	Tiamulin	NI (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Tylosin tartare base	NI (1)	NI (1)	1/11 (9.09	0/1 (100)
	Tulathromycin	NI (1)	NI (1)	1/11 (9.09	0/1 (100)
Folate inhibitor	Sulfadimethoxine	8-512 (1) / NI (1)	≥ 512 (1)	2/11 (18.18)	1/2 (50.00)
	Sulfamethoxazole-trimethoprim	0.25-256 (1) / 20-320 (1) / NI (1)	≥ 4 (1) / NI (2)	3/11 (27.27)	3/3 (100)
	Sulfametoxazol	NI (1)	≥ 4 (1)	1/11 (9.09)	1/1 (100)
	Sulfisoxazol	0.25-256 (1)	≥ 512 (1)	1/11 (9.09)	0/1 (100)
	Sulphonamide	8-1024 (1) / 16-2048 (1) / NI (1)	≥ 64 (1) / ≥ 256 (1) / ≥ 512 (1)	3/11 (27.27)	2/3 (66.66)
	Trimethoprim	0.25-32 (1) / 0.5-32 (1) / ≤0.062-512 (1)	≥ 2 (1) / > 2 (1) / NI (1)	3/11 (27.27)	1/3 (33.33)
Tetracyclines	Tetracycline	0.125-512 (1) / 0.25-256 (1) /0.5-64 (1) / 1-64 (1) / NI (2)	≥ 8 (2) / ≥ 16 (4)	6/11 (54.54)	4/6 (66.66)
	Chlortetracycline	NI (1)	NI (1)	1/11 (9.09)	1/1 (100)
	Oxytetracycline	NI (1)	NI (1)	1/11 (9.09)	1/1 (100)
Glycylcyclines	Tigecycline	0.5-8 (1)	NI (1)	1/11 (9.09)	0/1 (0.00)
Nitrofurans	Nitrofurantoin	0.5-16 (1)	NI (1)	1/11 (9.09)	0/1 (0.00)
	Nitrofurazone	0.5-64 (1)	≥ 128 (1)	1/11 (9.09)	NI/1
Polymyxin	Polymyxin B	0.25-16 (1)	≥ 32 (1)	1/11 (9.09)	NI/1
	Colistin	2-4 (1)	≥ 2	1/11 (9.09)	0/1 (0.00)

MIC = minimum inhibitory concentration, NI = not informed.

carbapenems, and tetracyclines (4.92%, 3/61), nitrofurans, phenicols, and polymyxins (3.28%, 2/61), and quinolones, lincosamides, monobactam, and glycylcyclines (1.64%, 1/61).

Among cephalosporins, ceftiofur was the main antimicrobial tested, present in five studies (45.45%, 5/11), followed by cefotaxime, cefoxitin, ceftazidime, and ceftriaxone, in two studies each (18.18%, 2/11), and cefazolin, cephalothin, cefuroxime, cefquinome, and cefepime, in one study each (9.09%, 1/11). Ampicillin was the main antimicrobial tested among penicillins, present in 10 studies (90.90%, 10/11), followed by ticarcillin in two (18.18%, 2/11). Besides ampicillin, eight other penicillins were tested in one study each: co-amoxiclav, amoxicillin/clavulanic acid, ampicillin/sulbactam, penicillin, piperacillin, piperacillin/tazobactam, and ticarcillin/clavulanic acid (9.09%, 1/11).

Regarding aminoglycosides, gentamicin was the most tested antimicrobial, present in 72.72% (8/11) of the studies, followed by streptomycin in 45.45% (5/11); kanamycin and neomycin in 27.27% each (3/11), spectinomycin in 18.18% (2/11); and apramycin, amikacin, and tobramycin in one study each (9.09%, 1/11). Fluoroquinolones were represented by ciprofloxacin in 27.27% (3/11); enrofloxacin and danofloxacin in 18.18% (2/11); and enoxacin, moxifloxacin and oxolinic acid in one study (9.09%, 1/11). About folate inhibitors, sulfamethoxazole/trimethoprim, sulphonamide, and trimethoprim were present in three studies each (27.27%, 3/11). At the same time, sulfadimethoxine was present in two (18.18%, 2/11), and sulfametoxazol and sulfisoxazol were present in only one study each 9.09% (1/11). Carbapens were represented by imipenem in two studies (18.18%, 2/11); ertapenem and meropenem in one study each (9.09%, 1/11). Tetracycline was the main antimicrobial tested among tetracyclines (54.54%, 6/11), while chlortetracycline and oxytetracycline were tested in only one study each (9.09%, 1/11).

For the phenicols, chloramphenicol and florfenicol were tested in six studies each (54.54%, 6/11). Nitrofurantoin and nitrofurazone, belonging to the nitrofurans class, were present in one study each (9.09%, 1/11), while polymyxins were represented by polymyxin B and colistin, both present in one study each as well (9.09%, 1/11). Quinolones, monobactam, lincosamides, and glycylcyclines classes were represented by one antimicrobial each: nalidixic acid (36.36%, 4/11), aztreonam (18.18%, 2/11), clindamycin (9.09%, 1/11), and tigecycline (9.09%, 1/11), respectively.

Antimicrobial resistance genotypes prospected by PCR

Seventeen studies (30.36%, 17/56) assessed antimicrobial resistance genes in virulent *E. coli* strains isolated from the intestinal tract of calves. The genes evaluated are associated with resistance against aminocoumarins, aminoglycosides, carbapenems, cephalosporins, cephamycins, diaminopyrimidines, macrolides, monobactam-cephalosporins, monobactams, phenicols, quinolones, quinolones-macrolides, sulfonamides-macrolides-cephalosporins, sulfonamides, and tetracyclines.

Aminoglycosides were the class with the highest number of different genes (17) associated with resistance, followed by the class of quinolones with 12 genes researched and tetracyclines with eight genes identified. The classes with fewer evaluated genes were aminocoumarin, carbapenems, and cephamycins, each with one or two assessed genes. Detailed information on genes searched by antimicrobial

class, as well as those identified in the selected articles, is shown in Table 5.

The studies that used phenotypic and molecular methods to assess virulence and resistance in *E. coli* strains isolated from the intestinal tract or feces of calves are shown in Figure 4.

DISCUSSION

The systematic review aimed to provide reliable data about the AMR among pathogenic *Escherichia coli* strains isolated from calves worldwide. However, the analysis of the selected studies showed important gaps in the information regarding the methodology used for antimicrobial susceptibility tests, such as the absence of quality control strains for the assays, no information on antimicrobial concentration tested, as well as poor report of the breakpoints or halo diameter criteria used to classify the strains as resistant or susceptible to antimicrobials. The absence of this critical information compromises the reliability of the results observed in some studies, in addition to impairing the performance of more robust analysis (meta-analysis) on these data and thereby the drawing of strong inferences, since key data were missing.

However, despite the lack of important data in some papers included, this systematic review judiciously analyzed the included studies using several criteria of eligibility, which allowed the generation of information of great relevance to the proposed subject, although the assessment on global frequency of isolates resistant or susceptible to antimicrobials could not be performed. In general, the results demonstrated high resistance rates of *E. coli* strains to the main classes of antimicrobials recommended or used to treat gastrointestinal infections in calves, such as tetracyclines, penicillin, folate inhibitors (sulfamethoxazole-trimethoprim), aminoglycosides, phenicol and fluoroquinolones (Constable 2009). Not coincidentally, these antimicrobial classes, especially tetracyclines, penicillins, folate inhibitors, macrolides, and aminoglycosides, are among the most used in food-producing animals in the United States (Food and Drug Administration – FDA) and the European Union (*European Medicines Agency* – EMA 2015). Therefore, the alarming rates of AMR observed in pathogens from animal origin in the present study, as well as by others (Ohene Larbi et al. 2021, Jia et al. 2022), are strongly related to the use of drugs of medical importance for food-producing animals. Furthermore, the intense use of antimicrobials in animals intended for human consumption can also raise another concern associated with their residues or metabolites in meat, milk and eggs (Menkem et al. 2019, Treiber & Beranek-Knauer 2021).

In fact, among the 17 antimicrobial classes tested by the disk-diffusion method, including 77 different drugs, resistance was not observed for only seven antimicrobial bases. At the same time, for 10, information on AMR was not available. Likewise, among the studies that performed MIC, 15 different classes were tested, representing a total of 61 different antimicrobial drugs, from which only eight did not exhibit AMR, while for 15, information was not available. These findings emphasize the disturbing situation of the AMR among *E. coli* of animal origin, especially considering the great diversity of drugs and concentrations assessed, and call attention to their potential risks for animal and public health.

Disk diffusion was the most used technique, probably because this method is less expensive and laborious than the MIC method. However, a negative point of this method is that it only provides qualitative information (resistant, intermediate and susceptible), whereas when using MIC, it is possible to obtain qualitative and quantitative results, making it possible to determine the lowest concentration of the antimicrobial that will be able to inhibit bacterial growth. Furthermore, our findings also suggest a difficulty in treating these infections caused by *E. coli*, which can be even worse, taking into account that all studies included in the systematic review tested only strains that exhibited at least one virulence factor. A diversity of *E. coli* pathotypes with different potential to cause disease in animals and humans were investigated in the selected studies, with STEC (Shiga toxin *E. coli*) being the most searched, probably because cattle is the main reservoir for this pathotype and due to its clinical importance for humans (Coura et al. 2014, WHO 2015). In addition to the consequences for public health, several *E. coli* pathotypes identified in the selected papers are involved in gastrointestinal infections in calves, causing diarrhea and economic losses for animal production worldwide (Cho & Yoon 2014).

Regarding the spatial and temporal distribution of the papers included in the systematic review, they were published in the last 23 years, and India was the country with the highest number of publications, followed by Egypt, Spain and Brazil. India has the largest cattle herd in the world and is considered the epicenter of the global antimicrobial resistance crisis, due to consumption and inadequate production of antimicrobials (WHO 2015, Broom & Doron 2020). The spatial distribution of the studies points to a global interest in AMR in pathogenic *E. coli* isolated from calves, revealing an important participation of countries that are central players in livestock production (Fig. 3).

On the other hand, the temporal distribution of the selected papers shows a more recent concentration of studies on AMR among *E. coli* strains, which can be explained by the recent global increase of AMR among bacteria of medical importance (WHO 2022). In fact, the intensification of animal production and food trade globally can contribute to the spread of various forms of microbial resistance (Van Boeckel et al. 2015). In this sense, our

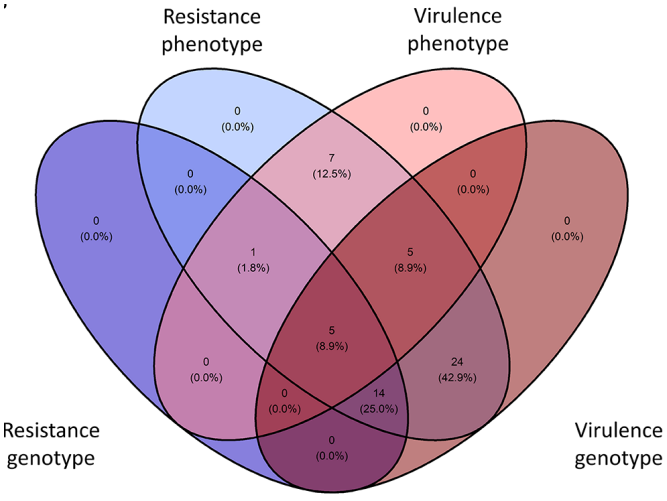

Fig. 4. Distribution of studies according to performance of resistance and virulence genotyping and phenotyping tests, selected by this systematic review on *Escherichia coli* isolated from the intestinal tract of calves and buffalo calves, published from 1982 to 2020.

Table 5. Frequency of prospection and identification of resistance genes in studies selected by this systematic review on *Escherichia coli* isolated from the intestinal tract or feces of calves, published from 1982 to 2020

Antimicrobial class	Gene/target	Resistance mechanism	N of studies that tested (%)	Identified
Aminocoumarin	*mdtABC-TolC*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
Aminoglycosides	*aac (3)-IV*	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aac (3)-II	Drug enzymatic inactivation	3/17 (17.64)	Yes
	aac (6)-Ib	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aadA	Drug enzymatic inactivation	2/17 (11.76)	NI
	aadA1	Drug enzymatic inactivation	5/17 (29.41)	Yes
	aadA5	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aadB	Drug enzymatic inactivation	3/17 (17.64)	Yes
	ampC	Drug enzymatic inactivation	3/17 (17.64)	Yes
	ant(2)-1	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aph(3")-Ib	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aph(3")-lc	Drug enzymatic inactivation	1/17 (5.88)	Yes
	aph(6)	Drug enzymatic inactivation	1/17 (5.88)	Yes
	blaCITM	Drug enzymatic inactivation	1/17 (5.88)	Yes
	kan	Work by binding to the bacterial 30S ribosomal subunit	1/17 (5.88)	Yes
	rmtB	Enzymatic modification of antibiotic target which	1/17 (5.88)	Yes
	strA	Enzymatic inactivation of antibiotic	3/17 (17.64)	Yes
	strB	Enzymatic inactivation of antibiotic	3/17 (18.75)	Yes
Carbapenems	*blaOXA-1*	Drug enzymatic inactivation	1/17 (5.88)	Yes
	IntI	Drug enzymatic inactivation	1/17 (5.88)	Yes
Cephalosporins	*blaCTX-M-14*	Drug enzymatic inactivation	1/17 (5.88)	Yes
	blaCTX	Drug enzymatic inactivation	1/17 (5.88)	Yes
	blaCTX-M-1	Drug enzymatic inactivation	1/17 (5.88)	Yes
	blaSHV	Drug enzymatic inactivation	2/17 (11.76)	Yes
	ctxM-1	Drug enzymatic inactivation	1/17 (5.88)	Yes
	ctxM-2	Drug enzymatic inactivation	1/17 (5.88)	Yes
	ctxM-9	Drug enzymatic inactivation	1/17 (5.88)	Yes
Cephamycin	*blaCMY*	Drug enzymatic inactivation	1/17 (5.88)	NI
	blaCMY-2	Drug enzymatic inactivation	1/17 (5.88)	Yes
Diaminopyrimidines	*dfrA-5*	Substitution of antibiotic action target	2/17 (11.76)	Yes
	drfA	Substitution of antibiotic action target	1/17 (5.88)	Yes
	drfA-1	Substitution of antibiotic action target	6/17(35.29)	Yes
	*drfA-14_*	Substitution of antibiotic action target	1/17 (5.88)	Yes
	drfA-17	Substitution of antibiotic action target	1/17 (5.88)	Yes
Macrolides	*erm(x)*	Enzymatic modification of antibiotic target	1/17 (5.88)	Yes
	ermB	Enzymatic modification of antibiotic target	1/17(5.88)	Yes
	macA	Antibiotic resistance via the transport of antibiotics	1/17 (5.88)	Yes
	macB	Antibiotic resistance via the transport of antibiotics	1/17 (5.88)	Yes
Monobactam, Cephalosporin	*tem-1*	Inactivation drug enzymatic modification	1/17 (5.88))	Yes
	tem-2	Inactivation drug enzymatic modification	1/17 (5.88)	Yes
Monobactams	*blaTEM*	Drug enzymatic inactivation	4/17 (23.52)	Yes
	blaTEM-1	Drug enzymatic inactivation	1/17 (5.88)	Yes
	blaTEM-1B	Drug enzymatic inactivation	2/17 (11.76)	Yes
Phenicols	*cat-1*	Drug enzymatic inactivation	2/17 (11.76)	Yes
	catA1	Drug enzymatic inactivation	2/17 (11.76)	Yes
	cmlA	Efflux proteins that pump antibiotic	5/17 (29.41)	Yes
	cmlA-1	Efflux proteins that pump antibiotic	1/17 (5.88)	NI
	floR	Efflux proteins that pump antibiotic	4/17 (23.52)	Yes
Quinolones	*acrEF-TolC*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	emrAB-OMF	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	gryA	Enzymatic modification of antibiotic target	1/17 (5.88)	Yes
	parC	Enzymatic modification of antibiotic target which	1/17 (5.88)	Yes

Antimicrobial class	Gene/target	Resistance mechanism	N of studies that tested (%)	Identified
Quinolones	*Oep*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	Onr	Protection of antibiotic action target	2/17 (11.76)	Yes
	QnrA	Protection of antibiotic action target	1/17 (5.88)	Yes
	QnrB	Protection of antibiotic action target	1/17 (5.88)	Yes
	QnrC	Protection of antibiotic action target	1/17 (5.88)	Yes
	QnrD	Protection of antibiotic action target	1/17 (5.88)	Yes
	QnrE	Protection of antibiotic action target	1/17 (5.88)	Yes
	QnrS	Protection of antibiotic action target	1/17 (5.88)	Yes
Quinolones, Macrolide	*mdtEF-TolC*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
Sulfonamides, Macrolides, Cephalosporin	*tolC-OpmH*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	mxAB-OprM	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	mexB	Antibiotic resistance via the transport of antibiotics	1/17 (5.88)	Yes
Sulfonamides	*sul1*	Substitution of antibiotic action target	8/17 (47.05)	Yes
	sul2	Substitution of antibiotic action target	4/17 (23.52)	Yes
	sul3	Substitution of antibiotic action target	2/17 (11.76)	Yes
Tetracyclines	*emrKY-TolC*	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	acrAB-TolC	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	tet(A)	Efflux proteins that pump antibiotic	9/17 (52.94)	Yes
	tet(B)	Efflux proteins that pump antibiotic	5/17 (29.41)	Yes
	tet(C)	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	tet(D)	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	tet(M)	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes
	tet(W)	Efflux proteins that pump antibiotic	1/17 (5.88)	Yes

NI = not informed.

results also showed that not only is resistance phenotypically present, but also genetically, with different antimicrobial resistance genes (ARG) identified in pathogenic *E. coli* strains isolated from calves. This is an important issue considering the ability of *E. coli* to exchange genetic material with numerous other bacteria, including microorganisms from normal microbiota (Braz et al. 2020). In this context, ARG can be transmitted from microorganisms of animal origin to humans and other animals (wildlife and domestic) through the contamination of different environments, representing a One Health risk (WHO 2021).

A limitation of this study was the inability to determine the exact frequency of antimicrobial-resistant or susceptible isolates (meta-analysis) due to the high heterogeneity among studies. In addition, there was an absence or poor description of crucial information regarding AMR, which hindered a more robust analysis of the results. These deficits identified in the studies hamper further discussion of the subject but highlight the need for studies such as ours to contribute to the prudent use of antimicrobials and on the importance of adopting a judicious methodology in scientific research, in order to guarantee the reliability of the study and the full use of the data generated.

CONCLUSION

This systematic review observed great heterogeneity in the criteria used by the studies to assess the antimicrobial resistance (AMR) of pathogenic *Escherichia coli* isolated from calves worldwide, revealing a low methodological quality in most of the selected papers. Nonetheless, despite that our results showed a high prevalence of AMR among the main classes used in the treatment of gastrointestinal infections caused by *E. coli*, especially tetracyclines, penicillin, folate inhibitors, macrolides, and aminoglycosides, besides a great pathogenic potential of the strains analyzed considering the virulence profiles and antimicrobial resistance genes (ARG) observed.

Acknowledgements.- The EMSD laboratory was supported by "Fundação de Amparo à Pesquisa de Minas Gerais" (FAPEMIG) (RED-00132-22), "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) and "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES). DACC, ACRF, MSG, ACTRBC and CRP are thankful to Capes for their fellowships. EMSD is also grateful to CNPq for her scholarship.

Conflict of interest statement.- The authors declare no conflict of interest.

Credit author statement.- Dircéia A.C. Custódio: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Amanda C.R. Ferreira: Writing – review & editing, Visualization, Validation, Methodology, Data curation, Conceptualization. Maysa S. Gonçalves: Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. Anna Cecília T.R.B. Costa: Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. Carine R. Pereira: Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. Fernanda M. Coura: Writing – review & editing, Funding acquisition, Formal analysis, Data curation. Andrey P. Lage: Review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. Geraldo Marcio da Costa: Review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. Elaine Maria S. Dorneles: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Data availability statement.- All data used and discussed are duly presented in the article.

REFERENCES

Andrade GI, Coura FM, Santos ELS, Ferreira MG, Galinari GCF, Facury Filho EJ, Carvalho AU, Lage AP, Heinemann MB. Identification of virulence factors by multiplex PCR in *Escherichia coli* isolated from calves in Minas Gerais, Brazil. Trop Anim Health Prod 2012; https://doi.org/10.1007/s11250-012-0139-8

Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, Shah T, Chaudhry TH, Rasool MH, Shahid A, Xueshan X, Baloch Z. Antibiotic resistance: one health one world outlook. Front Cell Infect Microbiol 2021; https://doi.org/10.3389/fcimb.2021.771510

Braz VS, Melchior K, Moreira CG. *Escherichia coli* as a multifaceted pathogenic and versatile bacterium. Front Cell Infect Microbiol 2020; https://doi.org/10.3389/fcimb.2020.548492

Broom A, Doron A. Antimicrobial resistance, politics, and practice in India. Qual Health Res 2020; https://doi.org/10.1177/1049732320919088

Buchanan R, Doyle M. Enterohemorrhagic *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli.* Food Technol 1997;51(10):69-76.

CDC. National Enteric Disease Surveillance: Shiga toxin-producing E. coli (STEC) Annual Report, 2017. Atlanta: Centers for Disease Control, Prevention, US Department of Health and Human Services, 2021.

Cho Y-I, Yoon K-J. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. J Vet Sci 2014; https://doi.org/10.4142/jvs.2014.15.1.1

Constable PD. Treatment of calf diarrhea: antimicrobial and ancillary treatments. Vet Clin N Am Food Anim Pract 2009; https://doi.org/10.1016/j.cvfa.2008.10.012

Coura FM, Lage AP, Heinemann MB. Patotipos de *Escherichia coli* causadores de diarreia em bezerros: uma atualização. Pesq Vet Bras 2014; https://doi.org/10.1590/S0100-736X2014000900001

EMA. Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), Fifth EVASC Report, 2015. (EMA/387934/2015).

Gu Z, Gu L, Eils R, Schlesner M, Brors B. *Circlize* implements and enhances circular visualization in R. Bioinformatics 2014; https://doi.org/10.1093/bioinformatics/btu393

Jia Y, Mao W, Liu B, Zhang S, Cao J, Xu X. Study on the drug resistance and pathogenicity of *Escherichia coli* isolated from calf diarrhea and the distribution of virulence genes and antimicrobial resistance genes. Front Microbiol 2022; https://doi.org/10.3389/fmicb.2022.992111

Joseph A, Cointe A, Kurkdjian PM, Rafat C, Hertig A. Shiga toxin-associated hemolytic uremic syndrome: a narrative review. Toxins 2020; https://doi.org/10.3390/toxins12020067

Kahlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, Rodloff A, Souss C-J, Steinbakk M, Soriano F, Stetsiouk O. European Committee on Antimicrobial Susceptibility Testing (EUCAST) technical notes on antimicrobial susceptibility testing. Clin Microbiol Infect 2006; https://doi.org/10.1111/j.1469-0691.2006.01454.x

Kolenda R, Burdukiewicz M, Schierack P. A systematic review and meta-analysis of the epidemiology of pathogenic *Escherichia coli* of calves and the role of calves as reservoirs for human pathogenic *E. coli.* Front Cell Infect Microbiol 2015; https://doi.org/10.3389/fcimb.2015.00023

Menkem ZE, Ngangom BL, Tamunjoh SSA, Boyom FF. Antibiotic residues in food animals: public health concern. Acta Ecologica Sinica 2019; https://doi.org/10.1016/j.chnaes.2018.10.004

Ohene Larbi R, Ofori LA, Sylverken AA, Ayim-Akonor M, Obiri-Danso K. Antimicrobial resistance of *Escherichia coli* from broilers, pigs, and cattle in the greater Kumasi metropolis, Ghana. Int J Microbiol 2021; https://doi.org/10.1155/2021/5158185

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, Chou R, Glanville J, Grimshaw JM, Hróbjartsson A, Lalu MM, Li T, Loder EW, Mayo-Wilson E, McDonald S, McGuinness LA, Stewart LA, Thomas J, Tricco AC, Welch VA, Whiting P, Moher D. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021; https://doi.org/10.1136/bmj.n71.

R-4.5.1 for Windows. 2022. Accessed on Aug. 22, 2022. https://cran.r-project.org/bin/windows/base/

Ray R, Singh P. Prevalence and implications of Shiga toxin-producing *E. coli* in farm and wild ruminants. Pathogens 2022; https://doi.org/10.3390/pathogens11111332

Sievert C, Parmer C, Hocking T, Chamberlain S, Ram K, Corvellec M, Despouy P, Brüggemann S. Package 'plotly'. Pacote R versão 4.6.0. 2017. Accessed on Aug. 22, 2022. https://CRAN.R-project.org/package=plotly

Treiber FM, Beranek-Knauer H. Antimicrobial residues in food from animal origin – a review of the literature focusing on products collected in stores and markets worldwide. Antibiotics 2021; https://doi.org/10.3390/antibiotics10050534

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R. Global trends in antimicrobial use in food animals. PNAS 2015; https://doi.org/10.1073/pnas.1503141112

WHO. Global antimicrobial resistance and use surveillance system (GLASS). World Health Organization, 2021. Accessed on Aug. 14, 2022. https://www.who.int/publications/i/item/9789240027336

WHO. Report signals increasing resistance to antibiotics in bacterial infections in humans and need for better data. World Health Organization, 2022.

WHO. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization, 2015; https://doi.org/10665/199350

Wickham H. ggplot2: elegant graphics for data analysis. 2ª ed. New York: Springer-Verlag; 2016. Accessed on Sep. 1, 2022. https://ggplot2.tidyverse.org

Supplementary material

Table S1. PRISMA 2020 Checklist

		TITLE	
Title	1	Systematic review on antimicrobial resistance in virulent *Escherichia coli* isolated from calves.	§1
		ABSTRACT	
Abstract	2	Provide relevant and structured information on the main findings regarding the systematic review, such as objectives, data source, type of studies, eligibility criteria, evaluation methods, results, limitations and conclusions.	§1
		INTRODUCTION	
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	§1, 2, 3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	§4

METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	§4
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	§2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	§3
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	§3
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and, if applicable, details of automation tools used in the process.	§6
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	§6
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	§6
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	§5
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	§5
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis – item #5).	§6
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics or data conversions.	§6
	13c	Describe any methods used to tabulate or visually display the results of individual studies and syntheses.	§6
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Not performed
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	Not performed
	13f	Describe any sensitivity analyses conducted to assess the robustness of the synthesized results.	Not performed
Reporting bias assessment	14	Describe any methods used to assess the risk of bias due to missing results in a synthesis (arising from reporting biases).	Not performed
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Not performed
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	§1 Figure 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Not performed
Study characteristics	17	Cite each included study and present its characteristics.	Supplementary Material Table S1
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Not performed
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	§1 to 18
Results of syntheses	20a	For each synthesis, briefly summarize the characteristics and risk of bias among contributing studies.	Not performed
	20b	Present the results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	§ 1 to 18
	20c	Present the results of all investigations of possible causes of heterogeneity among study results.	Not performed
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not performed
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Not performed
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Not performed

		DISCUSSION	
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	§1 to 5
	23b	Discuss any limitations of the evidence included in the review.	§1
	23c	Discuss any limitations of the review processes used.	Not performed
	23d	Discuss implications of the results for practice, policy, and future research.	§ 1 to 5
		OTHER INFORMATION	
Registration and protocol	24a	Provide registration information for the review, including the register name and registration number, or state that the review was not registered.	Not performed
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Not performed
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Not performed
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	§1
Competing interests	26	Declare any competing interests of review authors.	§1
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Not performed

From: Page et al. (2021).

Table S2. Combination of terms used at each database investigated within all the sections from papers (title, abstract and full text), as well as the number of articles found in the search performed on May 6th, 2020

Database	Combination of words	Results
CABI	((bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) AND (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND (Escherichia AND coli) AND (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") AND ("intestinal tract" OR diarrhea))	187 articles
Cochrane	((bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) AND (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND (Escherichia AND coli) AND (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") AND ("intestinal tract" OR diarrhea))	1 article
PubMed	((bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) AND (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND (*Escherichia* AND *coli*) AND (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") AND ("intestinal tract" OR diarrhea))	186 articles
SciELO	(bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) AND (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND (*Escherichia* AND **coli**) AND (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") AND ("intestinal tract" OR diarrhea)	6 articles
Scopus	TITLE-ABS- (bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) TITLE-ABS-KEY (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND TITLE-ABS-KEY (Escherichia AND coli) TITLE-ABS (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") TITLE-ABS-KEY ("intestinal tract" OR diarrhea)	162 articles
Web of Science	TS=((bovine* OR cattle OR calve* OR calf OR heifer* OR cow* OR herd* OR farm*) AND (enteropathogenic OR pathotypes OR "virulence genes" OR "virulence factors" OR virulence) AND (Escherichia AND coli) AND (antimicrobial OR antibiotic OR resistan* OR susceptibility OR "minimal inhibitory concentration" OR MIC OR "disc diffusion" OR "resistance genes" OR ARG OR "drug resistance") AND ("intestinal tract" OR diarrhea))	390 articles

MIC = minimum inhibitory concentration.

Table S3. Inclusion and exclusion criteria for selection of studies in this systematic review

Inclusion criteria	Exclusion criteria
Papers written in English, Spanish, French or Portuguese	Papers written in languages other than English, Spanish, French or Portuguese
Pathogenic Escherichia coli	Other microorganisms
Isolated from calves	Other animal species or other animal category other than calves
Virulence profile assessed by phenotypic or genotypic methods	No assessment of virulent factors
Assessment of in vitro antimicrobial susceptibility by phenotypic methods (MIC, disk diffusion or E-test)	No assessment of antimicrobial susceptibility
Original data	Full text not available
	Thesis, abstract, book chapter and reviews

MIC = minimum inhibitory concentration.

Table S4. Reference list of the 56 studies selected in this systematic review on antimicrobial resistance of *Escherichia coli* isolated from the intestinal tract of calves and buffalo calves, published from 1982 to 2020

First author (year)	Reference
Abdulgayeid (2015)	Abdulgayeid M, Foad S, Shahin H, Ibrahim MS. Molecular characterization of *Escherichia coli* isolated from buffalo calves in El-Behera Governorate. Alex J Vet Sci 2015; https://doi.org/10.5455/ajvs.202843
Aly (1996)	Aly AO, Abd El-Wahed ZH, Kohilo KH, El-Sheikh AR. Some studies on clinical, hematological and biochemical changes in diarrhoeic neonatal buffalo calves with reference to hygienic conditions. Assiut Vet Med J 1996; https://doi.org/10.21608/avmj.1996.183928
Arya (2008)	Arya G, Roy A, Choudhary V, Yadav MM, Joshi CG. Serogroups, atypical biochemical characters, colicinogeny and antibiotic resistance pattern of Shiga toxin-producing *Escherichia coli* isolated from diarrhoeic calves in Gujarat, India. Zoon Public Health 2008; https://doi.org/10.1111/j.1863-2378.2007.01093.x
Awosile (2020)	Awosile B, Reyes-Velez J, Cuesta-Astroz Y, Rodríguez-Lecompte JC, Saab ME, Heider LC, Keefe G, Sánchez J, McClure JT. Short communication: whole-genome sequence analysis of 4 fecal bla_{CMY-2}-producing *Escherichia coli* isolates from Holstein dairy calves. Res J Dairy Sci 2020; https://doi.org/10.3168/jds.2019-16560
Barigye (2012)	Barigye R, Gautam A, Piche LM, Schaan LP, Krogh DF, Olet S. Prevalence and antimicrobial susceptibility of virulent and avirulent multidrug-resistant *Escherichia coli* isolated from diarrheic neonatal calves. Am J Vet Res 2012; https://doi.org/10.2460/ajvr.73.12.1944
Borriello (2012)	Borriello G, Lucibelli M, De Carlo E, Auriemma C, Cozza D, Ascione G, Scognamiglio F, Iovane G, Galiero G. Characterization of enterotoxigenic *E. coli* (ETEC), Shiga-toxin producing *E. coli* (STEC) and necrotoxigenic E. *coli* (NTEC) isolated from diarrhoeic Mediterranean water buffalo calves (*Bubalus bubalis*). Res Vet Sci 2012; https://doi.org/10.1016/j.rvsc.2011.05.009
Bradford (1999)	Bradford PA, Petersen PJ, Fingerman IM, White DG. Characterization of expanded-spectrum cephalosporin resistance in E. *coli* isolates associated with bovine calf diarrhoeal disease. J Antimicrob Chemother 1999; https://doi.org/10.1093/jac/44.5.607
Bumunang (2019)	Bumunang EW, McAllister TA, Zaheer R, Polo RO, Stanford K, King R, Niu YD, Ateba CN. Characterization of non-O157 *Escherichia coli* from cattle faecal samples in the North-West Province of South Africa. Microorganisms 2019; https://doi.org/10.3390/microorganisms7080272
Cabal (2013)	Cabal A, Gómez-Barrero S, Porrero C, Bárcena C, López G, Cantón R, Gortázar C, Domínguez L, Álvarez J. Assessment of virulence factors characteristic of human *Escherichia coli* pathotypes and antimicrobial resistance in O157: H7 and non-O157: H7 isolates from livestock in Spain. Appl Environ Microbiol 2013; https://doi.org/10.1128/AEM.00537-13
Çabalar (2001)	Çabalar M, Boynukara B, Gülhan T, Ekín IH. Prevalence of rotavirus, *Escherichia coli* K99 and O157: H7 in healthy dairy cattle herds in Van, Turkey. Turkish J Vet Anim Sci 2001;25(2):191-196.
Das (2005)	Das SC, Khan A, Panja P, Datta S, Sikdar A, Yamasaki S, Takeda Y, Bhattacharya SK, Ramamurthy T, Nair GB. Dairy farm investigation on Shiga toxin-producing *Escherichia coli* (STEC) in Kolkata, India with emphasis on molecular characterization. Epidemiol Infect 2005; https://doi.org/10.1017/s0950268805004000
De Rauw (2019)	De Rauw K, Thiry D, Caljon B, Saulmont M, Mainil J, Piérard D. Characteristics of Shiga toxin producing-and enteropathogenic *Escherichia coli* of the emerging serotype O80: H2 isolated from humans and diarrhoeic calves in Belgium. Clin Microbiol Infect 2019; https://doi.org/10.1016/j.cmi.2018.07.023
De Rycke (1982)	De Rycke J, Le Roux P, Melik N, Raimbault P. Frequency of enteropathogenic K99+ ST+ *Escherichia coli* and rotaviruses in neonatal diarrhea of calves. Survey of a veterinarian's clientele in Sarthe. Ann Rech Vet 1982;12(4):403-411. PMid:6291442
Donaldson (2006)	Donaldson SC, Straley BA, Hegde NV, Sawant AA, DebRoy C, Jayarao BM. Molecular epidemiology of ceftiofur-resistant *Escherichia coli* isolates from dairy calves. Appl Environ Microbiol 2006; https://doi.org/10.1128/AEM.02770-05
Du (2004)	Du X, Xia C, Shen J, Wu B, Shen Z. Characterization of florfenicol resistance among calf pathogenic *Escherichia coli.* FEMS Microbiol Lett 2004; https://doi.org/10.1111/j.1574-6968.2004.tb09645.x
Du (2005)	Du X, Shen Z, Wu B, Xia S, Shen J. Characterization of class 1 integrons-mediated antibiotic resistance among calf pathogenic *Escherichia coli.* FEMS Microbiol Lett 2005; https://doi.org/10.1016/j.femsle.2005.03.021
ElAshmawy (2016)	ElAshmawy WR, Marouf S, Galal HM. Detection of virulence genes and antimicrobial resistance of bacterial isolates of diarrhea in newly borne buffalo calves. Res J Pharm Biol Chem Sci 2016;7(4):1728-1735.
Gamez (2006)	Gamez HAJ, Rigobelo EC, Fernandes SA, Marin JM, Avila FA. Diarréia bovina: estudo da etiologia, virulência e resistência a antimicrobianos de agentes isolados de bezerros da região de Ribeirão Preto–SP, Brasil. ARS Vet 2006; https://doi.org/10.15361/2175-0106.2006v22n1p22-30
Gharieb (2019)	Gharieb R, Fawzi E, Elsohaby I. Antibiogram, virulotyping and genetic diversity of *Escherichia coli* and *Salmonella* serovars isolated from diarrheic calves and calf handlers. Comp Immunol Microbiol Infect Dis 2019; https://doi.org/10.1016/j.cimid.2019.101367
Giammanco (2002)	Giammanco GM, Pignato S, Grimont F, Grimont PAD, Caprioli A, Morabito S, Giammanco G. Characterization of Shiga toxin-producing *Escherichia coli* O157:H7 isolated in Italy and in France. J Clin Microbiol 2002; https://doi.org/10.1128/jcm.40.12.4619-4624.2002
Gonzalez (1989)	González EA, Blanco J. Serotypes and antibiotic resistance of verotoxigenic (VTEC) and necrotizing (NTEC) *Escherichia coli* strains isolated from calves with diarrhoea. FEMS Microbiol Lett 1989; https://doi.org/10.1111/j.1574-6968.1989.tb03414.x
Gonzalez (2019)	Pasayo RAG, Sanz ME, Padola NL, Moreira AR. Phenotypic and genotypic characterization of enterotoxigenic *Escherichia coli* isolated from diarrheic calves in Argentina. Open Vet J 2019; https://doi.org/10.4314/ovj.v9i1.12
Gueler (2008)	Güler L, Gündüz K, Ok U. Virulence factors and antimicrobial susceptibility of *Escherichia coli* isolated from calves in Turkey. Zoon Public Health 2008; https://doi.org/10.1111/j.1863-2378.2008.01121.x
Gupta (2011)	Gupta N, Sharda R, Sharma V, Deshpande A, Udaykar A. Pathogenicity and antibiogram of *Escherichia coli* isolated from diarrhoeic cow calves. Indian J Field Vet 2011;7(2):1-4.
Hakim (2017)	Hakim AS, Omara ST, Syame SM, Fouad EA. Serotyping, antibiotic susceptibility, and virulence genes screening of *Escherichia coli* isolates obtained from diarrheic buffalo calves in Egyptian farms. Vet World 2017; https://doi.org/10.14202/vetworld.2017.769-773

First author (year)	Reference
Holland (1999)	Holland RE, Wilson RA, Holland MS, Yuzbasiyan-Gurkan V, Mullaney TP, White DG. Characterization of eae+ *Escherichia coli* isolated from healthy and diarrheic calves. Vet Microbiol 1999; https://doi.org/10.1016/S0378-1135(99)00013-9
Islam (2015)	Islam AKMA, Rahman M, Nahar A, Khair A, Alam MM. Investigation of pathogenic *Escherichia coli* from diarrheic calves in selective area of Bangladesh. Bangl J Vet Med 2015 https://doi.org/10.3329/bjvm.v13i1.23716
Iweriebor (2015)	Iweriebor BC, Iwu CJ, Obi LC, Nwodo UU, Okoh AI. Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in feces of dairy cattle farms in Eastern Cape of South Africa. BMC Microbiol 2015; https://doi.org/10.1186/s12866-015-0553-y
Khalifa (2019)	Khalifa E, Nossair MA, Ayoub MA, Hadad GA. Molecular characterization of diarrheagenic *E. coli* isolated from newly born calves and humans. Alexandria J Vet Sci 2019; https://doi.org/10.5455/ajvs.22828
Kohansal (2018)	Kohansal M, Asad AG. Molecular analysis of Shiga toxin-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from calves. Onderstepoort J Vet Res 2018; https://doi.org/10.4102/ojvr.v85i1.1621
Liao (2019)	Liao Z, Chen X, Li Z, Gao Y, Hu S. Molecular detection of virulence and drug resistance genes of pathogenic *Escherichia coli* from calves in Chongqing, China. Pakistan J Vet 2019;39(03):423-427.
Lupindu (2014)	Lupindu AM, Olsen JE, Ngowi HA, Msoffe PLM, Mtambo MM, Scheutz F, Dalsgaard A. Occurrence and characterization of Shiga toxin-producing *Escherichia coli* O157:H7 and other non-sorbitol–fermenting e. coli in cattle and humans in urban areas of Morogoro, Tanzania. VBZ 2014; https://doi.org/10.1089/vbz.2013.1502
Maciel (2019)	Maciel JF, Matte LB, Tasca C, Scheid DAR, Gressler LT, Ziech RE, Vargas AC. Characterization of intestinal *Escherichia coli* isolated from calves with diarrhea due to rotavirus and coronavirus. J Med Microbiol 2019; https://doi.org/10.1099/jmm.0.000937
Mahanti (2014)	Mahanti A, Samanta I, Bandyopadhyay S, Joardar SN, Dutta TK, Sar TK. Isolation, molecular characterization and antibiotic resistance of enterotoxigenic *E. coli (*ETEC) and necrotoxigenic *E. coli* (NTEC) from healthy water buffalo. Vet Arh 2014;84(3):241-250.
Manna (2006)	Manna SK, Brahmane MP, Manna C, Batabyal K, Das R. Occurrence, virulence characteristics and antimicrobial resistance of *Escherichia coli* O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. Lett Appl Microbiol 2006; https://doi.org/10.1111/j.1472-765X.2006.01975.x
Manzoor (2015)	Manzoor R, Shah MI, Asma-ul-husna, Wani SA, Pandit F, Dar PA, Mir MI. Prevalence, serodiversity and antibiogram of enterotoxigenic *Escherichia coli* (ETEC) in diarrhoeic calves and lambs of Kashmir valley (J&K), India. J Appl Nat Sci 2015; https://doi.org/10.31018/jans.v7i1.635
Medina (2011)	Medina A, Horcajo P, Jurado S, De La Fuente R, Ruiz-Santa-Quiteria JA, Domínguez-Bernal G, Orden JA. Phenotypic and genotypic characterization of antimicrobial resistance in enterohemorrhagic *Escherichia coli* and atypical enteropathogenic E. *coli* strains from ruminants. J Vet Diagn Invest 2011; https://doi.org/10.1177/104063871102300114
Mercado (2004)	Mercado EC, Rodriguez SM, D'Antuono AL, Cipolla AL, Elizondo AM, Rossetti CA, Malena R, Mendez MA. Occurrence and characteristics of CS31A antigen-producing *Escherichia coli* in calves with diarrhoea and septicaemia in Argentina. J Vet Med Series B 2003; https://doi.org/10.1046/j.1439-0450.2003.00610.x
Mohamed Ou Said (2019)	Mohamed Ou Said A, Contrepois M, Der Vartanian M, Girardeau JP. Factors and markers of virulence of *Escherichia coli* strains isolated from diarrhea in calves aged 4-45 days in Algeria. Rev Elev Méd Vét Pays Trop 1994;47(2):169-175. PMid:7863065
Montso (2019)	Montso PK, Mlambo V, Ateba CN. The first isolation and molecular characterization of Shiga toxin-producing virulent multi-drug resistant atypical enteropathogenic *Escherichia coli* O177 serogroup from South African cattle. Front Cell Infec Microbiol 2019; https://doi.org/10.3389/fcimb.2019.00333
Nizza (2010)	Nizza S, Mallardo K, Marullo A, Iovane V, De Martino L, Pagnini U. Antibiotic susceptibility of haemolytic *E. coli* strains isolated from diarrhoeic faeces of buffalo calves. Ital J Anim Sci 2010;9(1):134-137.
Orden (1999)	Orden JA, Ruiz-Santa-Quiteria JA, García S, Cid D, De La Fuente R. *In vitro* activities of cephalosporins and quinolones against *Escherichia coli* strains isolated from diarrheic dairy calves. Antimicrob Agents Chemother 1999; https://doi.org/10.1128/aac.43.3.510
Orden (2000)	Orden JA, Ruiz-Santa-Quiteria JA, García S, Cid D, De La Fuente R. *In vitro* susceptibility of *Escherichia coli* strains isolated from diarrhoeic dairy calves to 15 antimicrobial agents. J Vet Med Series B Infect Dis Vet Public Health 2000; https://doi.org/10.1046/j.1439-0450.2000.00356.x
Pereira (2011)	Pereira RVV, Santos TMA, Bicalho ML, Caixeta LS, Machado VS, Bicalho RC. Antimicrobial resistance and prevalence of virulence factor genes in fecal *Escherichia coli* of Holstein calves fed milk with and without antimicrobials. J Dairy Sci 2011; https://doi.org/10.3168/jds.2011-4337
Pervez (2018)	Pervez A, Anjum FR, Bukhari AA, Anam S, Rahman SU, Arshad MI. Isolation and virulence genes characterization of diarrheagenic *Escherichia coli* from Calves. Pak Vet J 2018; https://doi.org/10.29261/pakvetj/2018.042
Rigobelo (2006)	Rigobelo EC, Gamez HJ, Marin JM, Macedo C, Ambrosin JA, Ávila FA. Virulence factors of *Escherichia coli* isolated from diarrheic calves. Arq Bras Med Vet Zootec 2006; https://doi.org/10.1590/S0102-09352006000300003
Shahrani (2014)	Shahrani M, Dehkordi FS, Momtaz H. Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biol Res 2014; https://doi.org/10.1186/0717-6287-47-28
Sharma (2017)	Sharma RK, Taku AK, Malik A, Bhat MA, Javed R, Badroo GA, Kour A. Molecular characterization and antimicrobial profiling of *Escherichia coli* isolates from diarrheic calves. Indian J Anim Sci 2017; https://doi.org/10.56093/ijans.v87i12.79782
Smith (1988)	Smith HW, Huggins MB. The influence of plasmid-determined and other characteristics of enteropathogenic *Escherichia coli* on their ability to proliferate in the alimentary tracts of piglets, calves and lambs. J Med Microbiol 1978; https://doi.org/10.1099/00222615-11-4-471
Srivani (2019)	Srivani M, Reddy YN, Subramanyam KV, Lakshman M, Kavitha KL, Ramanipushpa RN. Prevalence, molecular characterization and antimicrobial resistance of necrotoxigenic *Escherichia coli* in diarrhoeic buffalo calves. IAVMI 2019; https://doi.org/10.5958/0974-0147.2019.00006.0

First author (year)	Reference
Srivani (2017)	Srivani M, Narasimha Reddy Y, Subramanyam KV, Ramakoti Reddy M, Srinivasa Rao T. Prevalence and antimicrobial resistance pattern of Shiga toxigenic *Escherichia coli* in diarrheic buffalo calves. Vet World 2017; https://doi.org/10.14202/vetworld.2017.774-778
Umpiérrez (2017)	Umpiérrez A, Bado I, Oliver M, Acquistapace S, Etcheverría A, Padola NL, Vignoli R, Zunino P. Zoonotic potential and antibiotic resistance of *Escherichia coli* in neonatal calves in Uruguay. Microbes Environ 2017; https://doi.org/10.1264/jsme2.ME17046
Valat (2012)	Valat C, Auvray F, Forest K, Métayer V, Gay E, Garam GP, Madec J-Y, Haenni M. Phylogenetic grouping and virulence potential of extended-spectrum-β-lactamase-producing *Escherichia coli* strains in cattle. Appl Environ Microbiol 2012; https://doi.org/10.1128/AEM.00351-12
Valat (2014)	Valat C, Forest K, Auvray F, Métayer V, Méheut T, Polizzi C, Gay E, Haenni M, Oswald E, Madec J-Y. Assessment of adhesins as an indicator of pathovar-associated virulence factors in bovine *Escherichia coli.* Appl Environ Microbiol 2014; https://doi.org/10.1128/AEM.02365-14
Vargas Júnior (2017)	Vargas Júnior SF, Cunha RC, Pereira DIB, Botton SÁ, Ladeira SRL, Lucia Júnior T, Sallis ESV. Identification of virulence factors of *Escherichia coli* isolates from fecal samples of calves in southern Brazil. Acta Sci Vet 2017; https://doi.org/10.22456/1679-9216.80441
Verdier (2012)	Verdier K, Nyman A, Greko C, Bengtsson B. Antimicrobial resistance and virulence factors in *Escherichia coli* from Swedish dairy calves. Acta Vet Scand 2012; https://doi.org/10.1186/1751-0147-54-2

Table S5. Detailed information of the 56 studies selected by this systematic review on antimicrobial resistance of *Escherichia coli* isolated from the intestinal tract of calves and buffalo calves, published from 1982 to 2020

First author (year)	Country	Period	Sample	Type study	Population	Age of animals	N of samples	Diarrhea frequency	AMR test	AMR test reference	N of virulent isolates
Abdulgayeid (2015)	Egypt	NI	Rectal swabs	Sectional	Buffalo calves	< 6 months	193	56.99	Disk diffusion	CLSI or Eucast	95
Aly (1996)	Egypt	NI	Feces	Sectional	Buffalo calves	2-4 months	38	100	Disk diffusion	CLSI or Eucast	12
Arya (2008)	India	2004-2005	Rectal swabs	Sectional	Calves	< 2 months	46	100	Disk diffusion	CLSI or Eucast	41
Awosile (2020)	Canada	2014-2015	Rectal swabs	No design	Calves	< 2 weeks	4	0.00	MIC	CLSI or Eucast	4
Barigye (2012)	USA	2010	Feces	Sectional	Buffalo calves	< 2 weeks	97	100	MIC	Other	23
Borriello (2012)	Italy	2006-2009	Intestinal content	Sectional	Calves	< 4 weeks	314	100	Disk diffusion	CLSI or Eucast	65
Bradford (1999)	USA	2006-2009	Feces	No design	Calves	NI	NI	100	Disk diffusion / MIC	CLSI or Eucast	10
Bumunang (2019)	South Africa	1996	Feces	Sectional	Calves	NI	600	NI	Disk diffusion	CLSI or Eucast	NI
Cabal (2013)	Spain	2015-2017	Feces	No design	Calves	NI	NI	0.00	MIC	CLSI or Eucast	68
Çabalar (2001)	Turkia	NI	Rectal swabs	Sectional	Calves	NI	59	15.25	Disk diffusion	Bauer et al. (1966)	1
Das (2005)	India	2001-2002	Feces	Sectional	Calves	NI	111	NI	Disk diffusion	CLSI or Eucast	13
De Rauw (2019)	Belgium	1987-2009-2015	NI	No design	Calves	16 days-2.5 months	NI	100	Disk diffusion	NI	9
De Rycke (1982)	France	1980	Feces	Sectional	Calves	NI	NI	NI	Disk diffusion	Other	10
Donaldson (2006)	USA	2003	Feces	Sectional	Calves	1-9 weeks	96	100	Disk diffusion/ MIC	CLSI or Eucast	10
Du (2005)	China	NI	NI	No design	Calves	NI	NI	100	MIC	CLSI or Eucast	13
Du (2004)	China	1982-1988	NI	No design	Calves	NI	NI	100	MIC	CLSI or Eucast	9
ElAshmawy (2016)	Egypt	NI	Feces	Sectional	Buffalo calves	1 day - 2 month	120	66.66	Disk diffusion	CLSI or Eucast	18
Gamez (2006)	Brazil	2001 – 2002	Feces	Sectional	Calves	< 3 months	200	100	Disk diffusion	Bauer et al. (1966)	53
Gharieb (2019)	Egypt	NI	Feces	No design	Calves	1-3 weeks	80	100	MIC	CLSI or Eucast	8

First author (year)	Country	Period	Sample	Type study	Population	Age of animals	N of samples	Diarrhea frequency	AMR test	AMR test reference	N of virulent isolates
Giammanco (2002)	Italy	NI	Feces	No design	Calves	NI	37	NI	Disk diffusion	Other	37
Gonzalez (1989)	Spain	NI	Feces	No design	Calves	< 30 days	289	100	Disk diffusion	Other	84
Gonzalez (2019)	Argentina	2014-2015	Feces	No design	Calves	2-10 days	NI	100	Disk diffusion	Bauer et al. (1966)	5
Gueler (2008)	Turkia	2001-2006	Feces	No design	Calves	< 2 months	NI	62.5	Disk diffusion	Other	66
Gupta (2011)	India	NI	NI	No design	Calves	NI	NI	100	Disk diffusion	Bauer et al. (1966)	NI
Hakim (2017)	Egypt	NI	Feces	No design	Buffalo calves	NI	58	100	Disk diffusion	CLSI or Eucast	14
Holland (1999)	USA	NI	Feces	Sectional	Calves	< 3 months	215	53.02	Disk diffusion	Bauer et al. (1966)	63
Islam (2015)	Bangladesh	2014	Feces	Sectional	Calves	6 days-2 months	100	100	Disk diffusion	Bauer et al. (1966)	2
Iweriebor (2015)	South Africa	NI	Feces	Sectional	Calves	NI	400	0.00	Disk diffusion	CLSI or Eucast	95
Khalifa (2019)	NI	2016	Feces	Sectional	Calves	< 3 months	100	100	Disk diffusion	CLSI or Eucast	9
Kohansa (2018)	Iran	2015-2016	Rectal swabs	Sectional	Calves	< 30 days	540	100	Disk diffusion	CLSI or Eucast	71
Liao (2019)	China	NI	Feces	Sectional	Calves	NI	30	100	Disk diffusion	CLSI or Eucast	18
Lupindu (2014)	Tanzania	2010-2012	Feces	Sectional	Calves	NI	446	NI	Disk diffusion	CLSI or Eucast	10
Maciel (2019)	Brazil	2014-2015	Feces	Case control	Calves	21-60 days	60	50.00	Disk diffusion	CLSI or Eucast	9
Mahanti (2014)	India	NI	Feces	Sectional	Buffalo calves	NI	363	0.00	Disk diffusion	CLSI or Eucast	25
Manna (2006)	India	2003	Feces	Sectional	Calves	1-3 months	79	100	Disk diffusion	Bauer et al. (1966)	11
Manzoor (2015)	India	NI	Feces	Sectional	Calves	< 4 months	NI	100	Disk diffusion	NI	6
Medina (2011)	Spain	1993-2005	Feces	No design	Calves	NI	NI	100	Disk diffusion	CLSI or Eucast	24
Mercado (2004)	Argentina	1995-200	Feces	No design	Calves	< 3 months	NI	100	Disk diffusion	Bauer et al. 1966	12
Mohamed Ou Said (2019)	Egypt	2015-2016	Feces	Sectional	Calves	≤ 3 months	56	46.42	Disk diffusion	CLSI or Eucast	6
Montso (2019)	South Africa	2017	Feces	Sectional	Calves	NI	780	NI	Disk diffusion	Bauer et al. (1966)	NI
Nizza (2010)	Italy	2006-2008	Feces	Sectional	Buffalo calves	≤ 30 days	169	100	Disk diffusion	CLSI or Eucast	94
Orden (1999)	Spain	1993-1995	Feces	No design	Calves	≤ 3 months	NI	100	MIC	CLSI or Eucast	137
Orden (2000)	Spain	1993-1995	Feces	No design	Calves	≤ 3 months	NI	100	MIC	CLSI or Eucast	137
Pereira (2011)	USA	2009	Feces	Sectional	Calves	2 days	117	47.86	Disk diffusion	CLSI or Eucast	117
Pervez (2018)	Pakistan	NI	Rectal swabs	Sectional	Calves	3 months	28	100	Disk diffusion	Other	2
Rigobelo (2006)	Brazil	2001-2002	Feces	Sectional	Calves	< 3 months	200	100	Disk diffusion	CLSI or Eucast	NI
Shahrani (2014)	Iran	2010-2011	Feces	Sectional	Calves	2-30 days	8241	100	Disk diffusion	Bauer et al. (1966)	419
dSharma (2017)	India	2013-2015	Feces	Sectional	Calves	≤ 3 months	350	100	Disk diffusion	NI	65

First author (year)	Country	Period	Sample	Type study	Population	Age of animals	N of samples	Diarrhea frequency	AMR test	AMR test reference	N of virulent isolates
Smith (1988)	Chile	NI	Rectal swabs	Sectional	Calves	≤ 10 days	77	100	Disk diffusion	Bauer et al. (1966)	32
Srivani (2019)	India	2014-2015	Feces	Sectional	Buffalo calves	< 3 months	375	100	Disk diffusion	Bauer et al. (1966)	34
Srivani (2017)	India	2014-2015	Feces	Sectional	Buffalo calves	< 3 months	375	100	Disk diffusion	CLSI or Eucast	106
Umpiérrez (2017)	Uruguay	2012-2014	Feces	No design	Calves	≤ 6 months	303	79.87	Disk diffusion	Bauer et al. (1966)	26
Valat (2012)	France	2006-2010	Feces	No design	Calves	NI	204	NI	Disk diffusion	NI	NI
Valat (2014)	France	2001-2012	Feces	No design	Calves	NI	259	NI	Disk diffusion	Bauer et al. (1966)	NI
Vargas Júnior (2017)	Brazil	NI	Feces	Sectional	Calves	≤ 6 months	40	37.50	Disk diffusion	Bauer et al. (1966)	12
Verdier (2012)	Sweden	2004-2005	Rectal swabs	Case control	Calves	≤ 1 months	95	58.90	MIC	CLSI or Eucast	NI

AMR = antimicrobial resistance, NI = not informed, MIC = minimum inhibitory concentration, CLSI = Clinical & Laboratory Standards Institute, Eucast = European Committee on Antimicrobial Susceptibility Testing.

Table S6. Evaluation of potential limitations and bias in the methodology of the 56 papers selected by this systematic review

First author (year)	Test MIC	Test disk diffusion/MIC	Reference protocol	Antibiotic	Breakpoint/Halo diameter
Abdulgayeid (2015)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Aly (1996)	0	Disk diffusion	CLSI or Eucast	Yes	Other
Arya (2008)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Awosile (2020)	Yes	MIC	CLSI or Eucast	Yes	CLSI or Eucast
Barigye (2012)	Yes	MIC	Other	No	Other
Borriello (2012)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Bradford (1999)	Yes	Disk diffusion/MIC	CLSI or Eucast	No	CLSI or Eucast
Bumunang (2019)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Cabal (2013)	0	MIC	CLSI or Eucast	Yes	CLSI or Eucast
Çabalar (2001)	0	Disk diffusion	Bauer et al. (1966)	No	Bauer et al. (1966)
Das (2005)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
De Rauw (2019)	0	Disk diffusion	NI	No	NI
De Rycke (1982)	0	Disk diffusion	Other	No	Other
Donaldson (2006)	Yes	Disk diffusion/MIC	CLSI or Eucast	Yes	CLSI or Eucast
Du (2005)	Yes	MIC	CLSI or Eucast	No	CLSI or Eucast
Du (2004)	Yes	MIC	CLSI or Eucast	No	CLSI or Eucast
ElAshmawy (2016)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Gamez (2006)	0	Disk diffusion	Bauer et al. (1966)	No	Bauer et al. (1966)
Gharieb (2019)	Yes	MIC	CLSI or Eucast	Yes	CLSI or Eucast
Giammanco (2002)	0	Disk diffusion	Other	No	Other
Gonzalez (1989)	0	Disk diffusion	Other	Yes	Other
Gonzalez (2019)	0	Disk diffusion	Bauer et al. (1966)	No	Bauer et al. (1966)
Gueler (2008)	0	Disk diffusion	Other	Yes	CLSI or Eucast
Gupta (2011)	0	Disk diffusion	Bauer et al. (1966)	No	Bauer et al. (1966)
Hakim (2017)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Holland (1999)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Islam (2015)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Iweriebor (2015)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Khalifa (2019)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Kohansal (2018)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Liao (2019)	0	Disk diffusion	CLSI or Eucast	Yes	NI
Lupindu (2014)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Maciel (2019)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast

First author (year)	Test MIC	Test disk diffusion/MIC	Reference protocol	Antibiotic	Breakpoint/Halo diameter
Mahanti (2014)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Manna (2006)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Manzoor (2015)	0	Disk diffusion	NI	Yes	NI
Medina (2011)	0	Disk diffusion	CLSI or Eucast	No	CLSI or Eucast
Mercado (2004)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Mohamed Ou Said (2019)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Montso (2019)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Nizza (2010)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Orden (1999)	Yes	MIC	CLSI or Eucast	No	CLSI or Eucast
Orden (2000)	Yes	MIC	CLSI or Eucast	No	CLSI or Eucast
Pereira (2011)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Pervez (2018)	0	Disk diffusion	Other	Yes	Other
Rigobelo (2006)	0	Disk diffusion	CLSI or Eucast	Yes	CLSI or Eucast
Shahrani (2014)	0	Disk diffusion	Bauer et al. (1966)	Yes	CLSI or Eucast
Sharma (2017)	0	Disk diffusion	NI	Yes	Bauer et al. (1966)
Smith (1988)	0	Disk diffusion	Bauer et al. (1966)	Yes	NI
Srivani (2019)	0	Disk diffusion	Bauer et al. (1966)	No	Bauer et al. (1966)
Srivani (2017)	0	Disk diffusion	CLSI or Eucast	No	Bauer et al. (1966)
Umpiérrez (2017)	0	Disk diffusion	Bauer et al. (1966)	No	CLSI or Eucast
Valat (2012)	0	Disk diffusion	NI	No	CLSI or Eucast
Valat (2014)	0	Disk diffusion	Bauer et al. (1966)	No	Other
Vargas Júnior (2017)	0	Disk diffusion	Bauer et al. (1966)	Yes	Bauer et al. (1966)
Verdier (2012)	Yes	MIC	CLSI or Eucast	Yes	CLSI or Eucast

MIC = minimum inhibitory concentration, NI = not informed, CLSI = Clinical & Laboratory Standards Institute, Eucast = European Committee on Antimicrobial Susceptibility Testing.

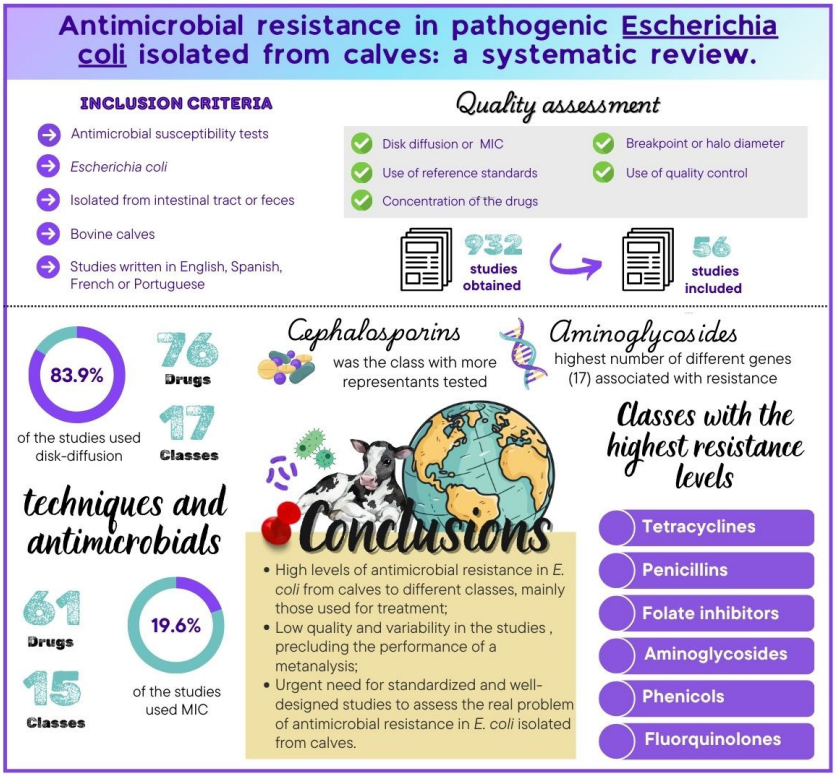

Fig. S1. Analysis of the main characteristics of the studies selected by this systematic review on *Escherichia coli* isolated from the intestinal tract of calves.