



Molecular Epidemiology and AMR Perspective of Diarrhoeagenic *Escherichia coli* in Africa: A Systematic Review and Meta-analysis

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Received: 17 June 2024 / Accepted: 5 September 2024
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Abstract

Introduction Diarrhoeagenic *Escherichia coli* (DEC) persistently challenges public health in Africa, contributing substantially to the diarrhoeal disease burden. This systematic review and meta-analysis illuminate the distribution and antimicrobial resistance (AMR) patterns of DEC pathotypes across the continent.

Methods The review selectively focused on pathotype-specific studies reporting prevalence and/or AMR of human-derived DEC pathotypes from African nations, excluding data from extra-intestinal, animal, and environmental sources and studies focused on drug and mechanism experiments. Pertinent studies were retrieved from SCOPUS, PubMed, and EBSCOhost, processed with Covidence, and screened in alignment with PRISMA guidelines.

Results The reviewed studies were predominantly hospital-based (80%) and paediatric-focused (91%), with a meagre 4.4% documenting DEC outbreaks. Seven DEC pathotypes were discerned, with Enteroaggregative *E. coli* (EAEC) being notably prevalent (43%, 95% CI 30–55%) and Enteroinvasive *E. coli* (EIEC) least prevalent (24%, 95% CI 17–32%). Identified non-susceptibilities were noted against essential antibiotics including ciprofloxacin, ceftriaxone, and ampicillin, while instances of carbapenem and Extended-Spectrum β -Lactamase (ESBL) resistance were scarce.

Conclusion Despite sporadic data on DEC prevalence and AMR in Africa, particularly in community settings, a palpable gap remains in real-time outbreak surveillance and comprehensive data documentation. Augmenting surveillance and embracing advancements in molecular/genomic characterisation techniques are crucial to precisely discerning DEC's actual impact and resistance continuum in Africa.

Keywords Diarrhoeagenic *Escherichia coli* · Africa · Food and water-borne diseases · Antimicrobial resistance

Abbreviations

AST	Antimicrobial susceptibility testing	ESBL	Extended spectrum beta-lactamase
CDC	Centers for disease control and prevention	ETEC	Enterotoxigenic <i>Escherichia coli</i>
CI	Confidence interval	EUCAST	European committee on antimicrobial susceptibility testing
CLSI	Clinical and laboratory standards institute	FBD	Foodborne diseases
CSV	Comma-separated values	GEMS	Global enteric multicenter study
DAEC	Diffusely-adhering <i>Escherichia coli</i>	MIC	Minimum inhibitory concentrations
EAEC	Enteroaggregative <i>Escherichia coli</i>	PCR	Polymerase chain reaction
EHEC	Enterohaemorrhagic <i>Escherichia coli</i>	PFT	Proportion with Freeman–Tukey double arc-sine transformation
EIEC	Enteroinvasive <i>Escherichia coli</i>	STEC	Shiga toxin-producing <i>Escherichia coli</i>
EPEC	Enteropathogenic <i>Escherichia coli</i>	VTEC	Vero cytotoxin-producing <i>Escherichia coli</i>
		WGS	Whole genome sequencing
		WHO	World health organisation

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1 Background

Diarrhoea is a significant public health concern in sub-Saharan Africa—with a high incidence due to factors like limited access to clean water and sanitation—leading to millions of cases annually—and is exacerbated by limited healthcare access, particularly among young children, HIV-positive individuals, and visitors from abroad [1–3]. Diarrhoea manifests as a symptom originating from infections induced by various organisms, including bacteria, viruses, and parasites, predominantly propagated through water contaminated with faeces. In low-income nations, Rotavirus and *Escherichia coli* are two predominant causative agents of moderate-to-severe diarrhoea, along with other pathogens like *Cryptosporidium* and *Shigella* [4, 5].

Despite recent studies in Africa revealing the problematic emergence of antimicrobial resistance (AMR) for common causes of diarrhoea, such as diarrhoeagenic *E. coli* [6], the full scope, distribution, molecular epidemiology, and antimicrobial resistance of diarrhoeagenic bacterial pathogens in the continent remain poorly understood, mainly because many cases go undetected, unreported, and, consequently, untreated [7]. A recent PulseNet International survey emphasised the absence of Whole Genome Sequencing (WGS) in foodborne surveillance outside the United States, Canada, and Europe, spotlighting significant disparities in resources and expertise across regions [8].

In response to this pressing need, the Africa Pathogen Genomics Initiative (PGI) of the Africa Centres for Disease Control and Prevention (Africa CDC) established a technical focus group of experts on Foodborne Diseases (FBD) in April 2022. A significant area of concern is *E. coli*, a member of the *Enterobacterales* family, which the World Health Organisation (WHO) has identified as one of twelve bacterial families that significantly threaten human health due to escalating antibiotic resistance [9]. The most vulnerable, such as young children, older adults, and those with compromised immunity or malnutrition, are at heightened risk. Key transmission factors include unhygienic practices, limited sanitation, and exposure to contaminated water sources for consumption and irrigation. The latter has been pinpointed as a significant factor in transmitting genes related to antibiotic resistance and increased pathogenicity [10].

While current research primarily analyses *E. coli* samples from diarrhoeic patients, there is a significant gap in our understanding of its prevalence in the broader community setting [1, 11]. The Global Enteric Multicenter Study (GEMS) provided insights into the genomic diversity of *E. coli*. Among others, their findings suggest the potential for certain strains to carry or acquire virulence genes typically associated with *E. coli* diarrhoeagenic pathotypes [5, 12,

13]. Beyond these insights, a fragmented understanding of *E. coli* pathotypes and their contribution to diarrhoeal diseases across the continent remains. Consequently, we lack a cohesive picture of this pivotal pathogen's epidemiology and associated antibiotic resistance in African settings.

Contrasting with developed regions such as the USA and Europe, which have robust *E. coli* surveillance systems [14–19], Africa contends with significant systemic challenges. The value of well-established FBD surveillance systems was exemplified by the United Kingdom's swift containment of a Shiga toxin-producing *Escherichia coli* (STEC) outbreak within five weeks using WGS [20]. As plans unfold for an African genomic FBD surveillance platform, understanding the prevalence, burden, and diversity of diarrhoeagenic *E. coli* from Africa becomes imperative. Addressing this gap is crucial, as it informs where to allocate resources and infrastructure.

Consequently, this systematic review examines the existing literature on diarrhoeagenic *E. coli* obtained from human stool samples of diarrhoeic cases in African healthcare settings and communities. Our objective is to elucidate the status of the main diarrhoeagenic *E. coli* pathotypes, viz. enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) and their antibiotic resistance profiles, which directly challenge primary therapeutic measures. By aggregating data until April 2023, this review sets the foundation for developing a comprehensive pan-African surveillance system that integrates WGS insights.

2 Methods

This systematic review utilised the Covidence (Veritas Health Innovation Ltd) data management system and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines [21]. The process encompassed importing journal articles from three databases into Covidence, followed by title and abstract screening (Fig. 1). After selecting the relevant articles, we proceeded with full-text screening and data extraction. The extracted data was then exported in the comma-separated values (CSV) file format for further analysis in Microsoft Excel, R Studio and JupyterLab.

2.1 Generation of Search Terms and Database Selection

The search terms were derived from common terms previously associated with our topic. We reviewed the reference

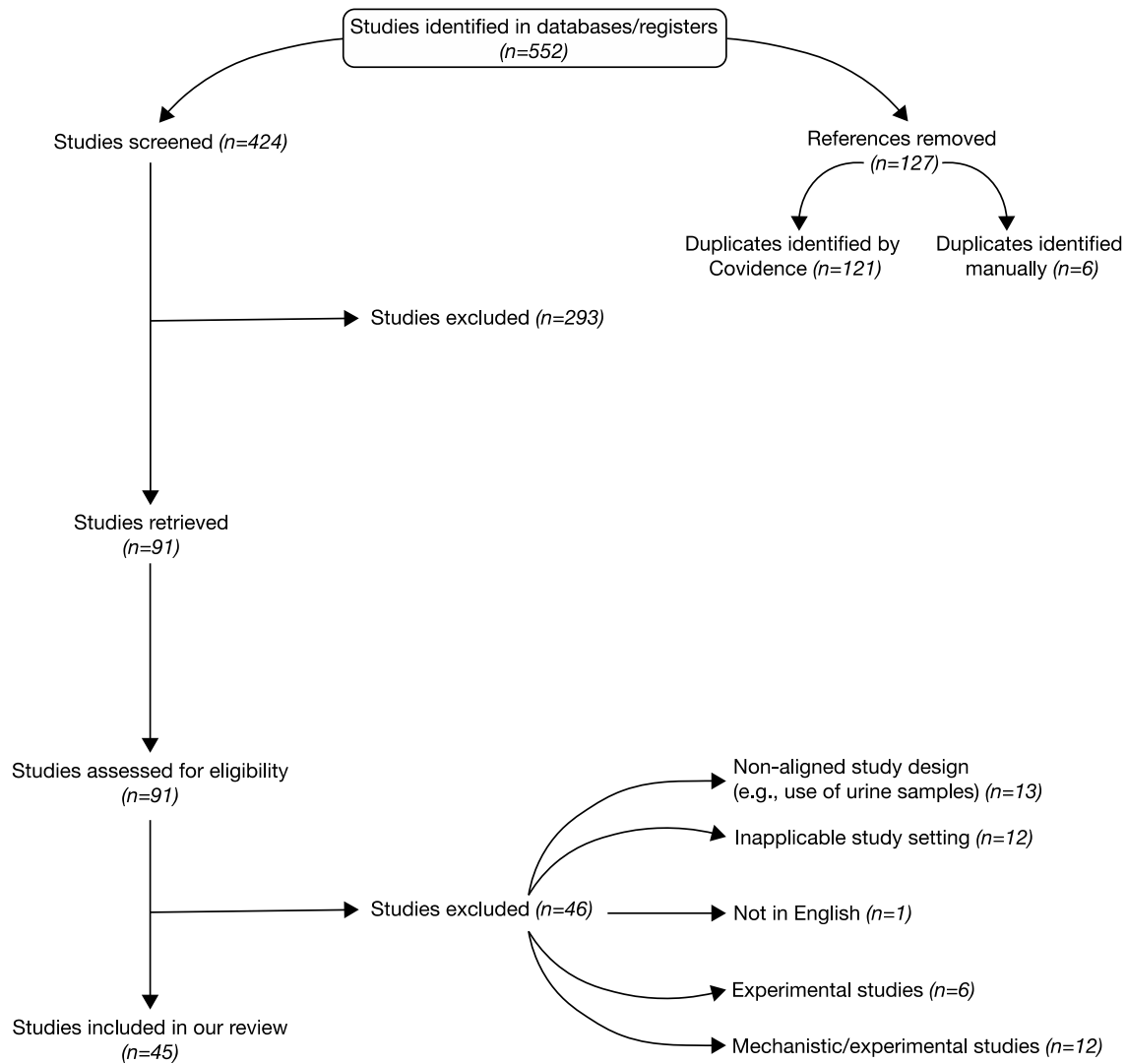


Fig. 1 A flow diagram depicting the flow of information through the various stages of the systematic review, drawn using PRISMA

sections of 30 articles pertinent to the molecular epidemiology of diarrhoeagenic *E. coli*. The journals and databases in which these references were published were then noted. The selection of databases for our literature search was based on the frequency of these journals. The keywords used for the search were determined by collating those from the 30 articles mentioned above and selecting the most frequently occurring keywords related to the molecular epidemiology of diarrhoeagenic *E. coli* in Africa.

2.2 Search Strategy

Database searches were conducted using Scopus, PubMed, and EBSCOhost Research Databases. The specific strategies for each are detailed in File S1.

2.3 Study Eligibility Criteria

We incorporated studies that specifically reported on the prevalence and or antimicrobial resistance patterns of diarrhoeagenic *E. coli* pathotypes derived from human sources. Still, we excluded samples from extra-intestinal sources and those that did not use molecular methods to confirm the pathotype.

Studies were excluded at the screening and full-text review stages if they were systematic or literature reviews, they exhibited an unclear study design, the articles were not written in English, or they sourced *E. coli* from non-human origins such as water, animals, soil, or food. Studies based on regions outside Africa (e.g., Europe, Asia, Americas, Australasia) were similarly excluded, along with papers reporting drug or mechanistic trials.

2.4 Risk of Bias and Methodological Quality Assessment

Two independent reviewers performed the risk of bias assessment and disagreements were solved by consensus. Each study's quality was scrutinised by two independent reviewers using a designated risk of bias and methodological quality assessment protocol for prevalence studies [22]. This tool was adapted onto the Covidence platform.

2.5 Title and Abstract Screening

Preliminary screening of the gathered studies was done based on their titles and abstracts. Two reviewers determined the eligibility of each study for inclusion. In cases where the reviewers' decisions clashed, a consensus was reached through discussion.

2.6 Full-Text Screening

At this juncture, the complete text of each article was meticulously perused by two reviewers to gauge its relevance. Any disagreement between the reviewers was settled through a mutual discussion to reach a final decision.

2.7 Data Extraction Strategy

We employed the Covidence software to devise a data extraction protocol tailored to accrue pertinent data about the antimicrobial resistance and prevalence of diarrhoeagenic *E. coli* pathotypes across Africa. This protocol was formulated and refined with the insights of four reviewers until a unanimous consensus was reached. During the extraction phase, each study was critically examined by two reviewers. Discrepancies in data extracted by the reviewers were addressed and resolved by a third reviewer's intervention.

2.8 Data Analysis

Upon the completion of data extraction, the results were transitioned into a comma-separated value (CSV) file format and integrated into Microsoft Excel for subsequent analysis. Statistical computations were predominantly executed using Python v3.10.9 via the JupyterLab interactive development environment v3.5.3. Data on pathotype prevalence was extracted and processed using Python's pandas library v2.0.3. The processed data, detailing the number of cases and the sample size for each study, was then passed to the R statistical environment for further analysis.

Using R's metafor package v4.2-0, a DerSimonian and Laird random-effects meta-analysis was performed. The metafor package computes effect sizes and associated variances, facilitating meta-analytic pooling of prevalence rates

across studies. For each study, the point estimate (proportion) of prevalence and its variance were computed using the `escalc` function, which utilises the proportion of cases (x_i) over the sample size (n_i) with the "PFT" measure.

Subsequently, the `rma` function from the metafor package was employed to compute the pooled random-effects estimate, taking care to account for between-study heterogeneity. This meta-analysis yielded effect sizes (or meta-estimates) for each study and an overall pooled effect size representing the cumulative prevalence estimate.

Pairwise comparisons were conducted to compare the prevalence estimates of various diarrhoeagenic *E. coli* pathotypes. The absolute differences in prevalence estimates between each pair of pathotypes were computed. To ascertain the significance of these differences, p -values were derived by comparing these differences to a normal distribution. Given the multiple comparisons, the Bonferroni correction was applied to adjust the significance level, ensuring the control of the family-wise error rate. A difference was deemed statistically significant if its associated p -value was lower than the Bonferroni-adjusted significance threshold.

Python library matplotlib v3.7.2 was used to generate a forest plot, displaying the prevalence rate for each study, along with the 95% confidence intervals. The overall pooled prevalence rate was distinctly highlighted to emphasise the aggregate findings of the analysis.

For data on antimicrobial resistance, the frequency and percentage of non-susceptible isolates for each antibiotic class were documented for studies where antibiograms were reported. For a selection of antibiotics, we used Stata v17 statistical (StataCorp) software to carry out a meta-analysis to determine the pooled resistance at the pathotype level.

We employed the Chi-Square and Fisher's Exact tests to investigate the statistical significance of observed antibiotic nonsusceptibility across different antibiotic classes. Each antibiotic class' observed frequencies were compared to expected frequencies based on the assumption of even distribution within that class. Specifically, when any expected frequency count in a class was less than or equal to five, the Fisher's Exact test was employed; this was especially pertinent for 2×2 tables but was extended here through a series of 2×2 tables, with the smallest p -value taken as representative. The Chi-Square test was employed in cases where all expected frequencies were above five. A p -value less than 0.05 indicated a significant departure from the expected distribution, thus suggesting that the observed frequencies were unlikely due to random variation alone.

2.9 Data Visualisation

All visualisations were created using Python's matplotlib library v3.7.2. The Set3 palette from seaborn library v0.12.2 was employed to ensure that distinct categories (like

pathotypes in our study) were easily distinguishable. For the distribution of pathotype-specific studies by country, a heatmap was used, where each cell in the heatmap displays the number of studies, with the colour intensity indicative of the quantity (the darker the shade, the higher the number). The methods used in the reviewed studies were visualised using a stacked bar chart, with the colour of each bar segment signifying a distinct method used in the studies in percentage terms. For the meta-analysis results, a forest plot-like visualisation was employed, where the estimated prevalence from different studies, along with their 95% confidence intervals, were presented using error bars. This format allowed for a clear comparison of prevalence estimates across studies and pathotypes.

3 Results

3.1 Geographical Distribution

Forty-five publications were reviewed for data extraction, spanning 18 African countries (Table S1). Most of the studies emerged from Kenya (24%) and South Africa (18%). Of the 45 articles, 76% (34/45) reported on EPEC, 69% (31/45) reported on EAEC and ETEC, 44% (20/45) reported on EIEC, 36% (16/45) reported on STEC/VTEC, 11% (5/45) reported on DAEC, and only 6% (3/45) reported hybrid strains.

An interesting observation from the geographical data was the pronounced concentration of reports of specific pathotypes in certain regions. Kenya, South Africa, and Nigeria emerged as areas where EPEC, EAEC, and ETEC studies commonly emanated—a pattern clearly illustrated in Fig. 2A.

3.2 Study Types

Most studies (43/45, 96%) comprised reports from non-outbreak settings, while only 4% (2/45) represented the retrospective use of outbreak samples. At the same time, 69% (31/45) were cross-sectional and 27% (12/45) were classified as case–control studies.

3.3 Population Characteristics in the Reviewed Studies

The human population samples covered a broad age spectrum. Still, the focus was predominantly on younger children despite many publications addressing more than one age group. One of the publications reported on neonates under 28 days, 31% (14/45) on infants under five years and 58% (26/45) on children under 18. Fewer reports (7/45, 16%) focused on adults over 18 years, including one focusing on

older people over 65, and 27% (12/45) did not specify the age groups.

In terms of specific population categories, 4% (2 out of 45) were in rural settings, 7% (3 out of 45) involved food handlers, and 2% (1 out of 45) each came from urban and peri-urban areas, with or without livestock. The rest were from unique reports, such as travellers' diarrhoea, low-income populations, a wedding party, and those suffering from underlying diseases.

3.4 Study Sites

All the examined publications reported on stool samples primarily collected in hospital settings at 67% (30 out of 45). In contrast, 13% (6 out of 45) of the studies collected samples from both hospital and community settings, and 16% (7 out of 45) were from community settings alone.

Of the 45 studies, all (100%) reported patients presenting with diarrhoea. Of these, 40% (18 out of 45) reported patients displaying severe signs of diarrhoea, including bloody diarrhoea (10%), vomiting (14%), and fever (14%).

3.5 Diagnostic Laboratory Techniques Utilised from the African Sourced Publications

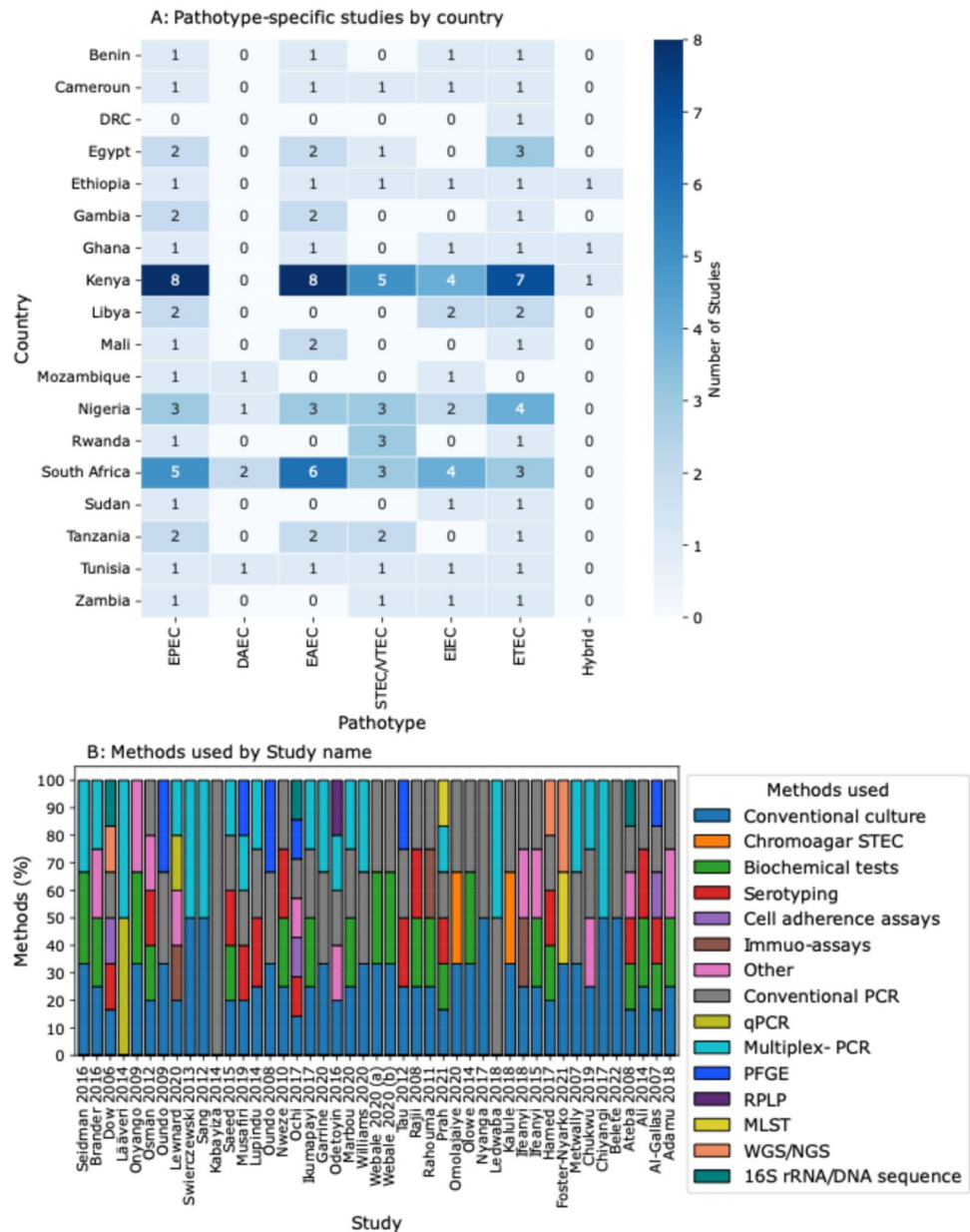
We explored the methodologies employed to detect diarrhoeagenic *E. coli*. From the 45 publications scrutinised, more than 15 analytic tools were identified (Fig. 2B). Most researchers (42/45; 93%) began their investigations with conventional culture techniques to isolate pathogens from clinical specimens. Subsequent screening and verification utilised a variety of approaches, including biochemical identification methods, conventional or multiplex PCR, and serotyping. Notably, only a few studies (3/45, 7%) employed sequencing tools, underscoring the limited capacity for advanced sequencing tools in food and waterborne research on the continent. Researchers often sought collaboration with national or international laboratories when specific tools were unavailable.

3.6 Prevalence of Diarrhoeagenic *E. coli* Pathotypes

Given that the reviewed publications did not consistently encompass all six main diarrhoeagenic pathotypes, our calculations for individual pathotypes included only those explicitly reported. Consequently, studies investigating specific pathotypes did not contribute prevalence data for other types not within their research scope. Our analysis highlighted EAEC as the most prevalent pathotype (43%; 95% CI 30–55%) (Fig. 3A), while STEC (Fig. 3E) and EIEC (Fig. 3F) presented the lowest prevalence at 28% (95% CI 14–42%) and 24% (95% CI 17–32%), respectively. Furthermore, ETEC, DAEC, and EPEC—inclusive of

Fig. 2 Overview of pathotype-specific studies by country and methodologies employed. Panel **A** (Pathotype-specific studies by country) illustrates the distribution of pathotype-specific studies across various countries. The countries are displayed on the y-axis, while distinct pathotypes are on the x-axis. The colour intensity, progressing from light to dark, represents an increasing number of studies, with the precise number annotated within each cell. Panel **B** (Methods used) details the techniques used across the studies reviewed. The methods are enumerated along the x-axis; each colour in the bars corresponds to a distinct method utilised in the research, as indicated by the colour-coded legend. The percentage (on the y-axis) denotes the prevalence of each method in each study.

PFGE pulsed field gel electrophoresis; *MLST* multilocus sequencing typing; *WGS* whole genomics sequencing; *NGS* next generation sequencing; *PCR* polymerase chain reaction; *qPCR*, quantitative polymerase chain reaction; *RFLP* restriction fragment length polymorphism; Other, includes techniques like haemolytic activities, verotoxicity tests, ELISA, transconjugation assays and the Colilert test



atypical EPEC— also emerged as notably prevalent pathotypes with prevalence rates of 36% (95% CI 27–45%), 36% (95% CI 16–57%), and 31% (95% CI 21–40%), respectively (Fig. 3B–D). Notably, there were only three reports of hybrid strains throughout the studies under review.

Comparative analyses of the prevalence of different pathotypes highlighted significant disparities among them (Table S2). EAEC exhibited the highest prevalence and was significantly more prevalent than ETEC, DAEC, EPEC, STEC, and EIEC, with differences in prevalence estimates ranging from approximately 6.56% to 18.61%. ETEC's prevalence was notably different from that of EPEC, STEC, and EIEC, though not significantly different from DAEC. Furthermore, DAEC showed a significantly distinct prevalence

from EPEC, STEC, and EIEC. EPEC and STEC differed insignificantly in prevalence. In contrast, there were noticeable differences between the prevalence of EPEC and EIEC and between STEC and EIEC.

3.7 Commonly Used Susceptibility Testing Techniques and Interpretive Standards

In our analysis of methodologies employed for assessing and interpreting antibiotic resistance across the selected studies, the Clinical and Laboratory Standards Institute (CLSI) guidelines emerged as the preferred framework. A substantial 90% (27/30) of the studies that detailed antibiotic resistance determinations opted for the CLSI guidelines. On the

other hand, the EUCAST guidelines found favour in only 10% (3/30) of the publications, with a number inclusive of reports following the directives of the Antibioqram Committee of the French Microbiological Society.

Regarding the antimicrobial susceptibility testing approach, the Kirby–Bauer disc diffusion method was the dominant technique, utilised by 73% (22/30) of the publications that reported non-susceptibility to an antibiotic. Another 23% (7/30) integrated the disc diffusion and micro-broth dilution methods. A minority, 10% (3/30), relied solely on the micro-broth dilution method. There was a solitary report of the VITEK system, with results closely mirroring those derived via the micro-broth dilution method.

Notably, only seven studies explored antibiotic-resistant genes, either as an exclusive method or in conjunction with the susceptibility assessment techniques mentioned earlier.

3.8 Antibiotic Resistance Among Diarrhoeagenic *E. coli*

Of 30 studies presenting antimicrobial resistance outcomes, a cumulative 602 antimicrobial resistance testing outcomes could be classified as antibiotic "non-susceptible", comprising 87% ($n = 521$) resistant and 14% ($n = 81$) intermediate resistant isolates (Table 1).

Among these, Quinolones surfaced as the most frequently encountered resistant class (p -value, $2.59E-11$), with a frequency of 105. Following closely were the Cephems, registering a frequency of 98 (p -value, $1.98E-13$), then Aminoglycosides and Penicillins, with frequencies of 79 and 76, respectively. Folate pathway inhibitors followed with a frequency of 59 (p -value, 0.0001).

Phenicol and Tetracyclines came next, with frequencies of 46 and 40, respectively. Non-susceptibility to Macrolides, β -lactam-inhibitor combinations, and Carbapenem classes were also observed. Notably, only four instances of extended-spectrum beta-lactamase (ESBL) phenotypes were observed (Table 1).

Our analysis revealed variable resistance patterns among the different *E. coli* pathotypes for the studies that reported antimicrobial susceptibility (AST) data (Figures S1–S5). Despite a prevalence of 36% for DAEC, none of the studies that reported on this pathotype, namely Garrine 2020, Omolajaiye 2020, Kalule 2018, and Ifeanyi 2015, provided data regarding antimicrobial susceptibility testing (ASTs). Hence, no available data exists on the prevalence of resistance among DAEC isolates. By contrast, STEC, ETEC, and EPEC isolates exhibited strikingly high resistance to ampicillin (the most frequently reported antibiotic among the reviewed studies) with prevalence rates and 95% confidence intervals (CIs) of 72% (13–100%), 73% (58–89%), and 72% (46–98%), respectively (Figures S1–S3), albeit with considerably wide confidence intervals, hinting at the

variability within the data and the small number of studies reporting AST data. On the other hand, pooled estimates for ampicillin resistance among EAEC and EIEC were 43.3% (95% CI 40–47%) and 43% (95% CI 36–51%), respectively. When assessing antimicrobial susceptibility across all diarrhoeagenic *E. coli* without distinguishing between individual pathotypes, the pooled resistance was as follows: 73% (95% CI 64–83%) for ampicillin, 32% (95% CI 19–46%) for gentamicin, 22% (95% CI 12–33%) for nalidixic acid, 14% (95% CI 8–20%) for ciprofloxacin, and 14% (95% CI 3–25%) for ceftriaxone (Fig. 4).

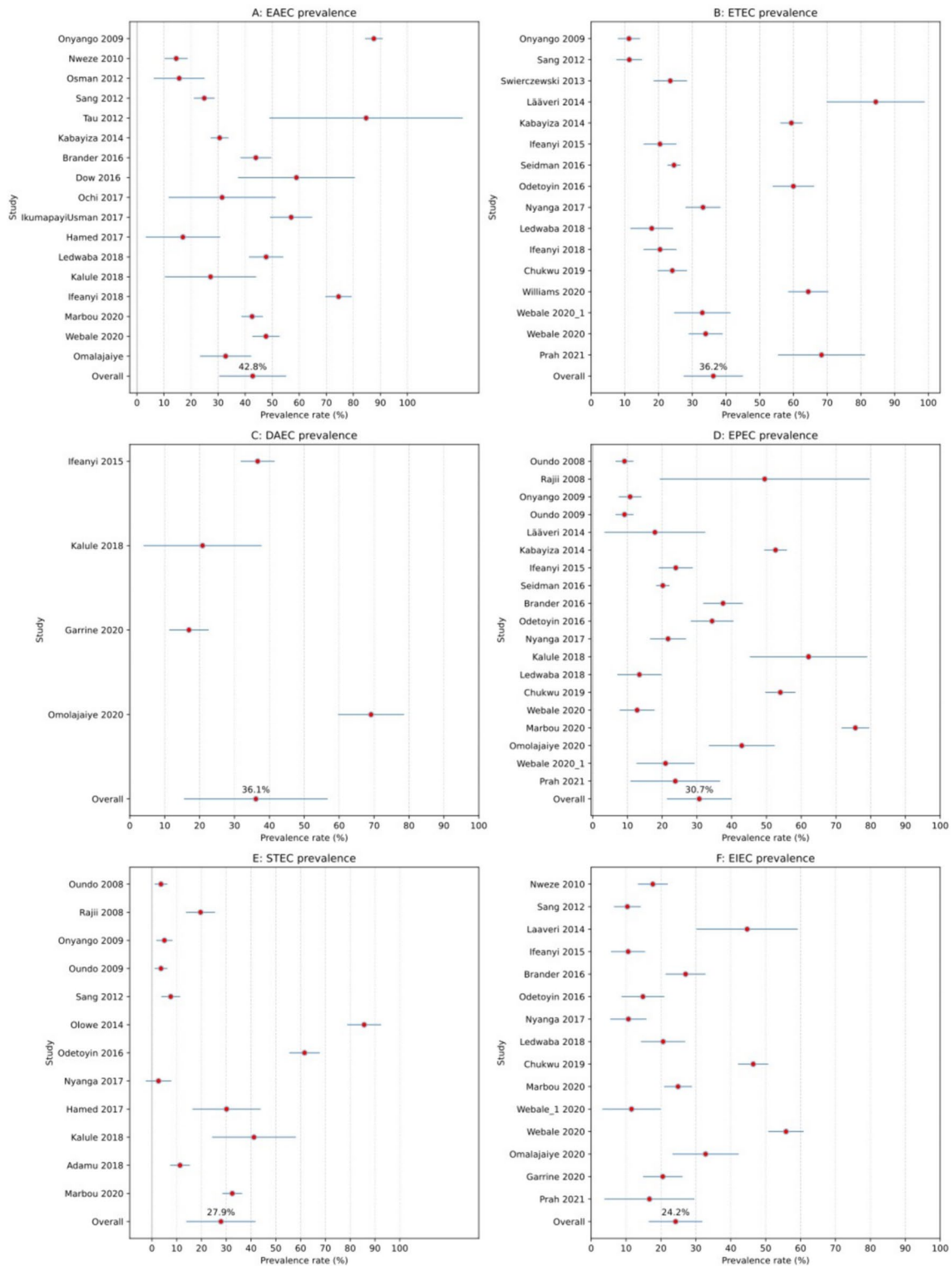
Of the 45 studies included in our review, only 9 (20%) reported multi-drug resistance (MDR) [23–31] (Table 1). Notably, one study identified four isolates resistant to all antibiotics tested, suggesting potential pan-drug resistance. However, since these isolates were not tested against all available antimicrobials, pan-drug resistance cannot be definitively confirmed [23]. Alarming, the same study reported that 71.4% of EHEC strains isolated from children were MDR.

Regarding the host and environmental factors associated with MDR in diarrhoeagenic *E. coli*, it was suggested that young age and unimproved sanitation may contribute to MDR due to increased exposure to fecal contamination [25]. High prevalence rates of MDR were reported for EPEC and EAEC, with these pathotypes being more likely to exhibit MDR than not [26]. Other studies reported MDR prevalence rates ranging from 53% to 91% in these two pathotypes [26–28, 31]. Interestingly, one study found that all diarrhoeagenic *E. coli* isolates from the stool of children were MDR, including EPEC [4], ETEC [6], EIEC [4], and EAEC [3, 24].

4 Discussion

This study reviewed Africa's prevalence and antimicrobial resistance patterns of diarrhoeagenic *E. coli* pathotypes. A significant proportion of reported studies emanated from Kenya, South Africa, and Nigeria. However, these numbers may not accurately reflect the actual disease prevalence across the continent. The disparity in the number of diarrhoeagenic *E. coli* studies underscores the varying diagnostic and surveillance practices across African nations. This can be attributed to the more developed healthcare infrastructures in Kenya, South Africa, and Nigeria [32], emphasising the need for enhanced surveillance and pathotype-specific interventions across Africa.

Our literature review identified EAEC and ETEC as the most frequently documented diarrhoeagenic *E. coli* pathotypes, contrasting with EIEC reported less frequently. These findings align with the GEMS study, which emphasised ETEC's significant role in moderate-to-severe diarrhea



among young children. While earlier studies predominantly acknowledged the impact of EAEC and ETEC on childhood diarrhea [1], our analysis reveals an increased recognition of ETEC, EAEC, and DAEC pathotypes. Subsequent findings from Ethiopia and Zambia corroborate our review's

outcomes, demonstrating a consistent pattern in the prevalence of these pathotypes [33–35].

Notably, our review suggests an underrepresentation of EHEC in literature, potentially overshadowed by pathogens causing dysentery that are more readily identified. In

Fig. 3 Prevalence of enteropathogenic bacterial pathotypes. The figure displays six distinct forest plots, each highlighting the prevalence of a specific enteropathogenic bacterial pathotype. Red circles represent the estimated prevalence rates from individual studies. Accompanying these markers, horizontal blue lines illustrate the 95% confidence interval (CI) for each respective rate. Each panel is dedicated to a different bacterial pathotype. Panel **A** elucidates the prevalence of Enteroaggregative *E. coli* (EAEC), while Panel **B** depicts Enterotoxigenic *E. coli* (ETEC). Similarly, Panels **C** through **F** focus on Diffusely adherent *E. coli* (DAEC), Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* (STEC), and Enteroinvasive *E. coli* (EIEC), respectively. The percentage value annotated above the 'Overall' marker indicates the cumulative meta-analysed prevalence rate associated with each pathotype. As a point of reference, a vertical grey line is drawn at the 0% prevalence rate mark, and dashed gridlines are included at regular intervals to facilitate a clearer understanding of the prevalence percentages

contrast, a 2017 study from an Iranian health center reported a striking prevalence of STEC at 50%, with EPEC and EHEC each accounting for 25% of cases [36]. This challenges the perceived rarity of EHEC's rarity in developing countries, hinting instead at a potential seasonal variation in prevalence.

Interestingly, only two studies in this systematic review pinpointed outbreaks triggered by a specific diarrhoeagenic *E. coli* pathotype, with one being attributed to a novel heat-stable enterotoxin-producing strain of Enteroaggregative *E. coli* [37]. The evident lack of real-time surveillance for food and waterborne pathogens in many regions likely obscures outbreaks' detection and actual frequency. Moreover, most studies in our review opted for culture-based diagnostic methods despite the evolution and optimisation of more sensitive genomic epidemiology techniques that could be adapted to low-resource settings [15, 16, 38, 39].

Our study highlights a significant gap in research on hybrid strains, which are often associated with more severe clinical outcomes than their non-hybrid counterparts, as highlighted by Santos et al. [40]. The paucity of research on these strains may stem from insufficient genomic capabilities in the region, hindering the identification of genotypic markers indicative of hybridity. This assertion is supported by the scant inclusion of genomics in *E. coli* pathotype surveillance across the studies we reviewed. The necessity for a more widespread application of genomics in diagnostic practices across Africa is evident. For instance, Akinlabi et al. (2023) conducted a study in Nigeria analysing diarrheic patient samples for *E. coli* pathotypes. Their comparison of gene identifications using established PCR primers with WGS revealed a high false-positivity rate for the *elt* gene due to its low specificity, leading to an overestimation of ETEC prevalence. In reality, many of these strains were identified as EAEC types through WGS. Misidentification between STEC and EHEC is also common [41], further underscoring the value of sequence data as diagnostic tools in enhancing disease management through accurate pathotype identification.

Despite the increasing reports of hybrid strains' virulence and multidrug-resistant properties in various countries, Zelelie et al. (2023) reported their presence at less than 0.5% in a combined study in Ethiopia and India [33], suggesting potential under-reporting or a genuinely lower prevalence in developing regions compared to developed countries.

Moreover, our analysis unveiled a trend of heightened resistance to pivotal antibiotics, notably Quinolones and Cephalosporins. The fragmented nature of the reports, limited our determination of prevalence rates of resistance for diarrhoeagenic *E. coli*. While our results provide important insights, it's crucial to note that this data only presents a snapshot of the situation, as they are based on a limited number of studies. We need dedicated and meticulously designed epidemiological studies to grasp the true prevalence of resistance. A comprehensive approach, grounded in surveillance and well-structured research, is paramount to understanding and combating the rising antimicrobial resistance tide. However, our findings warrant continuously enhanced efforts to prevent the increase and emergence of antimicrobial resistance on the continent [42].

A marked low resistance to ESBLs and third-generation Cephalosporins was observed among diarrhoeagenic *E. coli* in this study. While this might seem like a positive indication that the continent is relatively shielded from the global ESBL challenge, it is important to interpret these findings with caution. The low prevalence of ESBLs observed may not fully reflect the true state of AMR across the continent, as many studies investigating AMR did not genotype *E. coli* into specific pathotypes. Our study's search strategy focused on clinically relevant diarrhoeagenic *E. coli* pathotypes, which could have led to the exclusion of significant research targeting key resistance phenotypes, such as ESBL production, without assessing virulence genes to determine pathotypes. Such studies, among other methods, often used chromogenic media to isolate ESBL-producing *E. coli* with co-resistance to other commonly used antimicrobials, such as carbapenems [43, 44].

The observed low prevalence of ESBLs may also reflect the limited number of studies that specifically sought out ESBL determinants or utilised genomics to characterise resistance against this antibiotic class. Given the potential clinical implications, it is imperative that Africa sustains vigilant monitoring in this domain, ensuring that the continent remains proactive in the ongoing battle against antimicrobial resistance.

Of the four studies that documented ESBL production among diarrhoeagenic *E. coli*, one reported on the environmental correlates of antimicrobial resistance and noted that children whose caregivers used a shared pit latrine or who openly defecated were more likely to carry multidrug-resistant bacteria than those with flush or unshared toilets [45]. This underscores the need for broader, community-based

Table 1 Prevalent rates (counts) of non-susceptibility (intermediate and resistant phenotypes) to commonly used antibiotics

Antibiotic class	Count of non-susceptibility by antibiotic	Frequency (by class)	Percent	Cumulative	Test used	<i>p</i> -Value
Quinolones	Nalidixic acid, 32 (30.48%); Ciprofloxacin, 52 (49.52%); Norfloxacin, 20 (19.05%); Enrofloxacin, 1 (0.95%)	105	17.44	74.92	Chi-Square	2.5911E-11
Cephems	Ceftriaxone, 26 (26.53%); Cefotaxime, 20 (20.41%); Ceftazidime, 20 (20.41%); Cefpodoxime, 5 (5.10%); Cephalexin, 1 (1.02%); Cefuroxime, 14 (14.29%); Cephazolin, 5 (5.10%); Cefoxitin, 3 (3.06%); Cefepime, 2 (2.04%)	98	16.28	38.7	Chi-Square	1.9844E-13
Aminoglycosides	Gentamycin, 39 (49.37%); Streptomycin, 22 (27.85%); Kanamycin, 8 (10.13%); Neomycin, 1 (1.27%); Amikacin, 8 (10.13); Tobramycin, 1 (1.27%)	79	13.12	51.83	Chi-Square	1.8365E-16
Penicillins	Ampicillin, 61 (80.26%); Ticarcillin, 1 (1.32%); Ofloxacin, 12 (15.79); Amoxicillin, 2 (2.63%)	76	12.62	12.62	Chi-Square	1.7065E-27
Folate pathway inhibitors	Trimethoprim, 14 (23.73%); Trimethoprim + sulfamethoxazole, 35 (59.32%); Sulphamethoxazole, 10 (16.95%)	59	9.8	22.43	Chi-Square	0.00010417
Phenicol	Chloramphenicol, 46 (100%)	46	7.64	96.67	Chi-Square	
Tetracyclines	Tetracycline, 38 (95%); Oxycycline, 1 (2.50%); Doxycycline, 1 (2.50%)	40	6.64	89.03	Chi-Square	1.3686E-15
Macrolides	Erythromycin, 19 (55.88%); Clarithromycin, 5 (14.71%); Azithromycin, 10 (29.41%)	34	5.65	57.48	Chi-Square	0.01178207
β -lactam-inhibitor combinations	Amoxicillin-clavulanic acid, 23 (92.0%); Piperacillin-tazobactam, 2 (8.00%)	25	4.15	79.07	Chi-Square	2.6691E-05
Carbapenem	Ertapenem, 1 (5%); Meropenem, 11 (55%); Imipenem, 8 (40%)	20	3.32	82.39	Chi-Square	0.0192547
Lipopeptides	Colistin sulfate, 7 (100%)	7	1.16	98.33	Chi-Square	
Glycylcycline	Tigecycline, 6 (100%)	6	1	99.34	Chi-Square	
ESBL	ESBL, 4 (100%)	4	0.66	100	Fisher's Test	1
Nitrofurans	Nitrofurantoin, 3 (100.00%)	3	0.5	97.17	Fisher's Test	1

research on food and waterborne pathogens. Unfortunately, our review noted that most studies reported on children less than 18 years of age during a health crisis and that more than two-thirds of the samples studied were reported from hospital sites. However, the actual burden of the disease within a community is better represented by community samples.

The interplay between antibiotic resistance and the virulence of pathogenic bacteria, including *E. coli*, has garnered significant global concern [1, 46, 47]. While some previous studies have suggested reduced virulence among multidrug resistant *E. coli* isolates relative to sensitive strains [48], others have emphasised that the acquisition of antimicrobial resistance does not necessarily compromise microbial fitness [49].

Consistent with this notion, epidemiological data indicate that antibiotic resistance and virulence factor carriage are linked in *E. coli* populations in some community settings [50]. A related study showed that the expression of virulence factors led to the forming of an antibiotic-tolerant

subpopulation [51] and that antibiotic treatment may be selected for virulence [52]. In addition to drug resistance, treatment failure in using antibiotics in a clinical setting could be due to tolerance and/or persistence to antibiotics [53]. Importantly, community-based surveillance studies are pivotal, as evidenced by findings linking sanitation practices with antibiotic resistance patterns [45]. However, our review focused on younger populations and hospital-based studies, underscoring the need for broader, community-based research.

Accurate pathotype identification remains a challenge due to the complexities associated with conventional laboratory techniques. The predominant reliance on the disk diffusion method over minimum inhibition concentration (MIC) for antimicrobial testing introduces further complexity due to varying sensitivity levels. Notwithstanding these challenges, a noticeable trend toward employing more sensitive diagnostic methodologies has emerged, suggesting an optimistic trajectory for future African studies.

On this note, the recent endeavours by Africa CDC, particularly through the foodborne disease focus group, underscore the continent's readiness to embrace advanced surveillance platforms for tracking food and waterborne disease outbreaks. Leveraging high-resolution techniques incorporating genomics, such as whole-genome sequencing (WGS), will not only elevate the precision of outbreak detection but also revolutionise our understanding of disease spread and antimicrobial resistance patterns. This approach, if widely adopted, positions Africa at the forefront of combating food and waterborne pathogens and ensuring the health and safety of its populace.

In our systematic review of published data on diarrhoeagenic *E. coli* from the African continent's public health sector, we encountered significant challenges in data collation. The heterogeneity in study designs and methodologies resulted in fragmented outputs. Notably, a limited number of studies reported AST data, and where they occurred, antimicrobial resistance profile determinations utilised a diverse array of antibiotics. Furthermore, many studies reported concentrations not aligned with the CLSI or EUCAST breakpoint guidelines. This disparity underscores the pressing need for standardised testing, reporting, and interpretive guidelines tailored to Africa's unique demographics, geographies, and economic scales. Such standardisation would ensure reproducibility and establish a robust platform for historic surveillance, enabling timely assessment of risks to the healthcare sector across the continent.

4.1 Limitations

This review was meticulously designed with comprehensive search criteria to encompass a broad spectrum of studies on *E. coli* pathotypes. However, given the vast expanse of scientific literature on this topic, some relevant studies may have been inadvertently overlooked. We recognise that by focusing our search strategy on diarrhoeagenic *E. coli* pathotypes, we may have missed crucial literature that broadly reported on key antimicrobial resistance phenotypes, such as ESBL, without delineating the specific diarrhoeagenic pathotypes.

Furthermore, significant heterogeneity was observed among the studies reviewed, stemming from differences in study design, population demographics, geographical locations, and methods of pathotype identification. Such heterogeneity can inevitably influence the overall prevalence estimates. While rigorous meta-analytic techniques were employed to mitigate this variation, we acknowledge that the reported rates may not capture the complete picture. They might, in fact, reflect under-reporting due to various factors, including limited diagnostic capacities or logistical constraints in certain settings.

5 Conclusions

Our review of diarrhoeagenic *E. coli* research in Africa reveals considerable variability in study approaches, populations, and sampling sites, with Kenya, South Africa, and Nigeria emerging as hotspots for research into particular

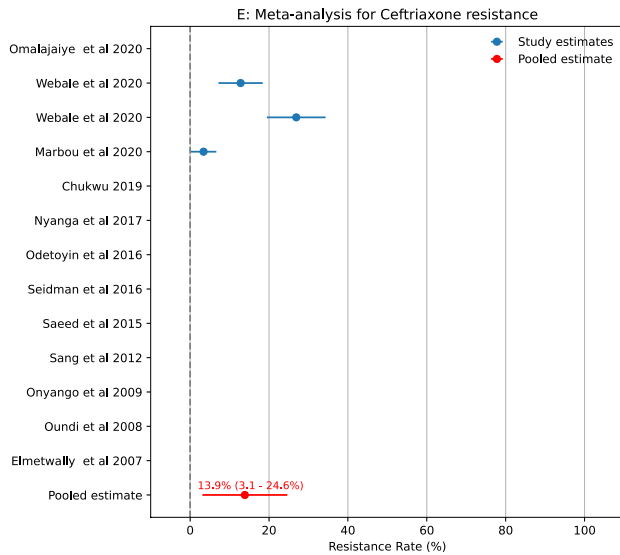
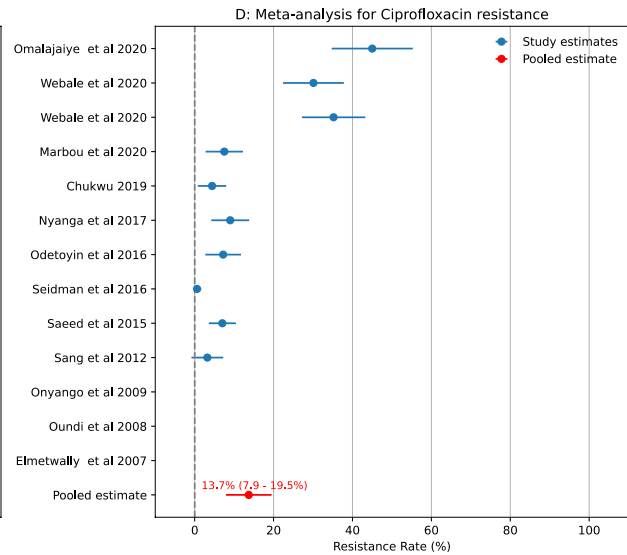
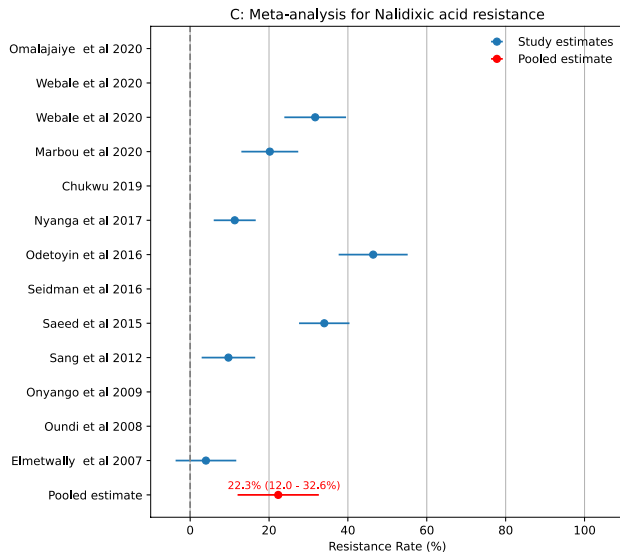
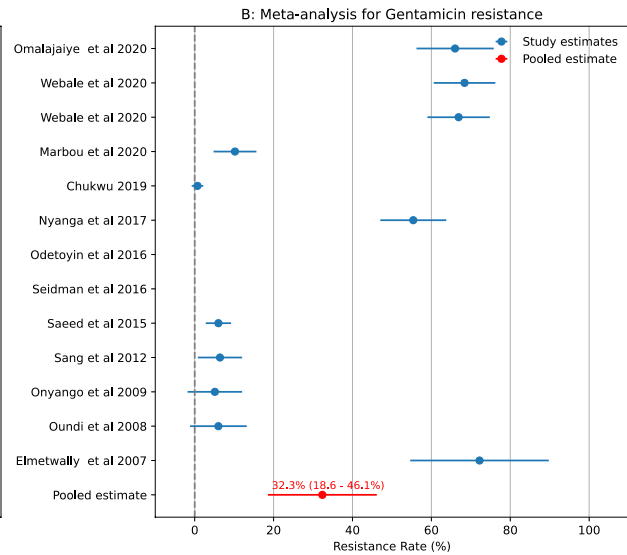
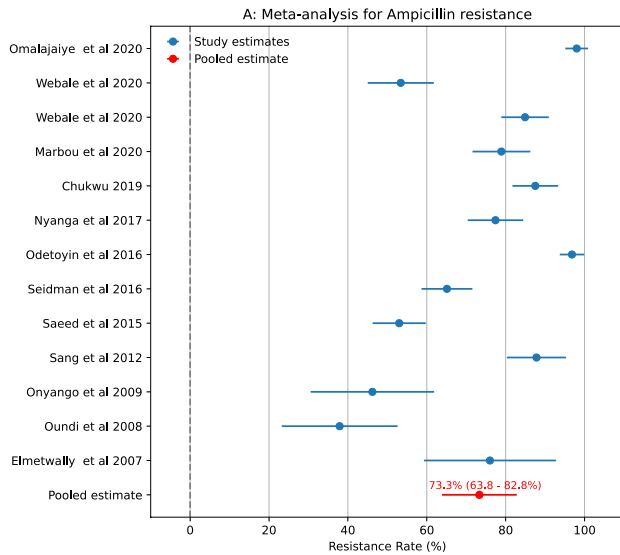


Fig. 4 Meta-analysis of antibiotic resistance prevalence in diarrhoeagenic *E. coli* across studies. This figure showcases a series of subplots, each dedicated to representing the prevalence of resistance to a particular antibiotic among all diarrhoeagenic *Escherichia coli* (DEC) strains. Each blue dot pertains to a specific study and displays the proportion of DEC samples in that study that exhibited resistance to the antibiotic under consideration. The horizontal position of each dot indicates the resistance percentage. The vertical position denotes the study from which the data originates. The error bars, extending from each dot, represent the 95% confidence interval (CI) of the resistance percentage for that study. The red dot in the first position of each subplot represents the pooled estimate of the resistance rate from a random effects model. The horizontal lines connected to the red dot indicate the 95% CIs of the pooled estimate. Beside each pooled estimate dot is a label specifying the exact resistance percentage and the corresponding 95% CIs

pathotypes. The research often centers on hospital samples and varies widely in detection methods and antibiotic resistance testing, particularly highlighting EPEC, EAEC, and ETEC pathotypes.

The predominance of EAEC and the scarcity of hybrid strain reports emphasise the need for enhanced surveillance and management. Alarming, there's a high resistance to antibiotics, notably to critical drugs like ciprofloxacin, signaling an urgent need for effective antibiotic stewardship, unified surveillance, and awareness campaigns for healthcare workers and the public.

Our analysis points to the widespread use of CLSI guidelines and the Kirby-Bauer disc diffusion method for antibiotic resistance testing, suggesting opportunities for standardising antimicrobial resistance measurement and reporting.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s44197-024-00301-w>.

Acknowledgements The authors wish to thank the Africa CDC for their support throughout this systematic review. We also thank Professor Kathryn E. Holt for her valuable time and insightful feedback on the draft manuscript.

Author Contributions Conceptualisation: JBK, LAB. Methodology and Software: JBK, LAB, DLB, FAD, EFN. Formal Analysis and Visualisation: JBK, LAB, DLB, FAB, EFN. Data curation: JBK, LAB, EFN. Writing—Original Draft Preparation: JBK, LAB, EFN, DLB, FAD. Review and approval of final manuscript: CM, AMS, AA, HK, GK, KY, JM, YJF, NS, AMK, PMKN, FC, SKT, and SIS.

Funding This review was generously funded by the African Union.

Availability of Data and Materials Data is provided within the manuscript or supplementary information files. All data generated or analysed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare that they have no competing interests.

Ethical Approval and Consent to Participate This review paper does not involve any original research with human or animal subjects. As such, no ethical approval was required for this study. The review was conducted and reported ethically, ensuring all sources are appropriately cited. No personal or sensitive data were used or shared in the preparation of this manuscript. Conflicts of interest, if any, have been disclosed, and appropriate acknowledgments have been made.

Consent for Publication Not applicable. This review article does not contain any individual person's data in any form (including individual details, images, or videos) that would require consent for publication.

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