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Prevalence of extended-spectrum beta-lactamase producing bacteria from animal origin: A systematic review and meta-analysis report from India

Suresh P. Kuralayanapalya[1], Sharanagouda S. Patil[1], Sudhakar Hamsapriya[1], Rajamani Shinduja[1], Parimal Roy[1], Raghavendra G. Amachawadi[2]*

1 ICAR-National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Yelahanka, Bengaluru, India, **2** Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States of America

* agraghav@vet.ksu.edu

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Abstract

Antimicrobial resistance (AMR) due to the emergence and spread of extended-spectrum beta-lactamase (ESBL) producing bacteria are becoming a serious global public health concern. This article aims to assess the overall prevalence of ESBLs among animals in India, with year-wise, zone-wise and species-wise stratification. Systematic search from PubMed, Google Scholar and J-Gate Plus was carried out and 24 eligible articles from 2013–2019 in India were retrieved. The R Open source Scripting software was used to perform statistical analysis. The overall prevalence of ESBLs among animals in India was 9%. The pooled prevalence of ESBLs in animals were 26, 11, 6 and 8% for north, east, south and central zones, respectively. The reported prevalence of ESBLs in animals were 12, 5, 8, 8, 12, 13 and 33% were reported for the years 2013, 2014, 2015, 2016, 2017, 2018, 2019 respectively. The species-wise stratified results showed a predominance of ESBL producing *Klebsiella pneumoniae* strains (11%) when compared to *Escherichia coli* and *Pseudomonas* spp. which were 7% and 5%, respectively. The prevalence data generated could be utilized in infection control and in antibiotic use management decisions for developing appropriate intervention strategies.

Introduction

Antimicrobial resistance (AMR) has been universally recognized as an emerging global problem to public health. Although the prevalence of AMR is sporadic, it is widespread in the Asian region. India, located in the southern part of Asia, marks a high, immeasurable burden of AMR among livestock due to poor documentation, sub-standard regulations with a shortfall in forbidding protocol enforcement [1]. This study aims to estimate the pooled prevalence of Extended-spectrum β-lactamases (ESBLs) in India by conducting systematic review and meta-analysis with 23 available research articles under epidemiological study design. Beta-Lactam

responsible for the content and writing of this article.

antimicrobial agents are the most favored class of antimicrobials for the treatment of bacterial infections, hence becoming the main cause of resistance to β-lactam antibiotics, globally [2]. Prevalence of ESBLs producing *Klebsiella* is becoming a major concern in China, Korea, Japan and India [3]. ESBLs enzymes are produced by the gram-negative bacteria to incur resistance against the β-lactams. *Klebsiella pneumoniae* and *Escherichia coli* are the main gram-negative bacteria producing ESBLs [4]. However *Proteus mirabilis*, *Enterobacter* spp., *Salmonella*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* also produce ESBLs to acquire resistance [5]. The incessant liability of gram-negative strains to a myriad β-lactams has begotten rapid and vigorous production and mutation of β-lactamases in these bacteria, hence, incurring resistance against the newly developed β-lactam antibiotics [2]. Treatment for these disease causing multidrug-resistant (MDR) organisms is a therapeutic challenge. The risk factors for developing infection with ESBL-producing organisms include indiscriminate and off-label use of antibiotics [6]. At present, animals without any recognized risk factor for multidrug-resistant organisms are found to have ESBL-producing organisms. Hence, diagnosis of ESBL-producing organisms has become vital [7]. MDRs are posing a treatment challenge, and a major cause of morbidity and mortality worldwide [1]. Unfortunately, India, being a developing country, does not have an adequate surveillance system that could track indiscriminate use or consumption of antibiotics in livestock populations. This meta-analysis will improve our understanding of the distribution of ESBLs in India. A set of similar events for which a study is conducted is called a population, in our study it refers to poultry, bovine and birds. The outcome of our study would indicate the prevalence of ESBLs by zone, year and species in India. It is a quantitative, epidemiological study designed to systematically assess the previous research studies to derive the conclusions of this research [8]. This study highlighted the prevalence of ESBL from the time period 2013–2019, with zone-wise and species-wise prevalence of ESBLs in India. A priori protocol was followed for this study with reference to a work done by Bulabula and co-workers [9]. To our knowledge, this is the first meta-analysis report from India on animals, which would aid in updating the national treatment guidelines for ESBL infections among animals.

Materials and methods

Literature search

A Systematic search was conducted in "Pub Med", "Google Scholar" and "J-Gate-Plus" databases from Jan 2013 to May 2019 using the search terms "ESBL", "prevalence", "India", "Animals", "Poultry", "Cattle" and "Bovine" in combinations. Bibliographies of eligible studies were also manually searched to identify additional significant articles. A comprehensive search was conducted to ensure none of the research were missed out. The search was restricted to articles published in English.

Study selection criteria

All the articles that described the frequency of ESBL producing pathogens among the total isolates from animal samples (clinical/healthy) were considered eligible and included in the study. The qualified articles described the specific laboratory methods used to identify the ESBL producing pathogen along with species of the ESBL producing organism (Table 1). All the enrolled studies were restricted to India. Review articles, case reports and outbreaks were excluded.

Data extraction

For consistency, data was extracted independently by two people from selected articles. The data extracted from qualified studies included year of publication, first author, location where

Table 1. Characteristics of studies included in the review.

Author and year of publication	State	Country	Sample type	Number of ESBL positive samples/Total number of samples (% prevalence)	Methodology	ESBL producing species
Bandyopadhyay et al. 2018 [25]	West Bengal	India	Bovine milk samples	12/424	PCR-based detection of major ESBL blaCTX-M-15 gene	ESBL producing K. pneumonie
Bhattacharya et al., 2015 [21]	West Bengal	India	Meat and meat products	2/80 (2.5%)	Combined Disc Diffusion Test	ESBL producing *E. coli*
Bhave et al., 2019 [28]	Maharashtra	India	Cloacal swabs of broilers	23/146(15.75%)	PCR-based detection of major ESBL genes (blaTEM, blaSHV, blaCTX-M)	ESBL producing E. coli
Bhoomika et al., 2016 [10]	Chhattisgarh	India	Chicken meat	2/65 (3.08%)	Multiplex-polymerase chain reaction for detection of bla_{TEM}, bla_{SHV},and blaCTX-M genes	ESBL genes in *E. coli*
Bhoomika et al., 2016 [10]	Chhattisgarh	India	Chevon meat	1/38 (2.63%)	Multiplex-polymerase chain reaction for detection of bla_{TEM}, bla_{SHV}, and blaCTX-M genes	ESBL genes in *E. coli*
Bhoomika et al., 2016 [10]	Chhattisgarh	India	Raw milk	6/73 (8.22%)	Multiplex-polymerase chain reaction for detection of bla_{TEM}, bla_{SHV}, and blaCTX-M genes	ESBL genes in *E. coli*
Brower et al., 2017 [12]	Punjab	India	Cloacal swabs from birds	305/1556 (19.60%)	Combination disk method and VITEK 2	ESBL producing *E. coli*
Chauhan et al., 2013 [13]	Himachal Pradesh	India	Raw milk samples from Doon valley	27/100 (27%)	Double disc diffusion method	ESBL producing *K. pneumoniae*
Das et al., 2017 [15]	West Bengal	India	Milk samples of subclinical mastitis infected cattle	24/50 (48%)	PCR-based detection of major ESBL genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M})	ESBL producing gram negative isolates
Dewangan et al., 2017 [16]	Chhattisgarh	India	Chevon meat	8/126 (6.35%)	Phenotypic detection of ESBL	ESBL producing *E. coli*
Dewangan et al., 2017 [16]	Chhattisgarh	India	Raw milk samples	8/104 (7.69%)	Phenotypic detection of ESBL	ESBL producing *E. coli*
Kar et al., 2015 [22]	West Bengal	India	Fecal samples from poultry	16/170 (9.41%)	Combination disc method and ESBL E-test	ESBL producing *E. coli*
Kar et al., 2015 [22]	West Bengal	India	Milk samples from cattle	2/135 (1.48%)	Combination disc method and ESBL E-test	ESBL producing *E. coli*
Karuppasamy et al., 2015 [23]	Mizoram	India	Raw milk samples	7/35 (20%)	Kirby-Bauer disc diffusion method	ESBL producing *E. coli* and *K. pneumoniae*
Koovapra et al., 2016 [40]	West Bengal	India	Bovine milk samples	7/159 (4.40%)	Combination disc diffusion test and ESBL Etest	ESBL producing *K. pneumoniae*
Koovapra et al., 2016 [40]	Jharkhand	India	Bovine milk samples	10/78 (12.82%)	Combination disc diffusion test and ESBL Etest	ESBL producing *K. pneumoniae*
Koovapra et al., 2016 [40]	Mizoram	India	Bovine milk samples	6/103 (5.82%)	Combination disc diffusion test and ESBL Etest	ESBL producing *K. pneumoniae*
Lalzampuia et al., 2013 [16]	Mizoram	India	Fecal samples of pigs	7/138 (5.07%)	PCR based detection of ESBLs genes	ESBL genes in *E. coli*
Lalzampuia et al., 2013 [17]	Mizoram	India	Fecal samples of poultry birds	4/102 (3.92%)	PCR based detection of ESBLs genes	ESBL genes in *E. coli*
Lalzampuia et al., 2014 [18]	Mizoram	India	Fecal samples of poultry birds	1/11 (9.09%)	PCR based detection of ESBLs genes	ESBL genes in *K. pneumoniae*
Mahanti et al., 2017 [14]	West Bengal	India	Cloacal swabs from healthy broiler, indigenous, and kuroiler birds	33/307 (10.75%)	PCR-based detection of major ESBL genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M})	ESBL producing *K. pneumoniae*
Mandakini et al., 2015 [24]	Mizoram	India	Fecal samples of piglets suffering from diarrhea	43/170 (25.29%)	Double disc synergy test	ESBL producing *E. coli*
Nirumapa et al., 2018 [26]	Uttar	India	Fecal samples of pigs	243/741(32.79%)	Double disc diffusion method and Hi-comb MIC test strip	ESBL producing *E. coli*

(*Continued*)

Table 1. (Continued)

Author and year of publication	State	Country	Sample type	Number of ESBL positive samples/Total number of samples (% prevalence)	Methodology	ESBL producing species
Raj et al., 2019 [29]	Karnataka	India	Food-animal environment	12/43(27.90%)	PCR-based detection of major ESBL blaCTX-M	ESBL producing E. coli
Rasheed et al., 2014 [19]	Telangana	India	Unpasteurized milk of buffalo	2/30 (6.67%)	Phenotypic Confirmatory Disc Diffusion Test	ESBL producing *E. coli*
Rasheed et al., 2014 [19]	Telangana	India	Raw chicken	0/30 (0%)	Phenotypic Confirmatory Disc Diffusion Test	ESBL producing *E. coli*
Rasheed et al., 2014 [19]	Telangana	India	Fresh raw meat of sheep	1/30 (3.33%)	Phenotypic Confirmatory Disc Diffusion Test	ESBL producing *E. coli*
Samanta et al., 2015 [31]	West Bengal	India	Samples from backyard and farmed poultry	23/360 (6.39%)	PCR-based detection of major ESBL genes (bla_{TEM}, bla_{SHV}, bla_{CTX-M})	ESBL producing *E. coli*
Sharif et al., 2017 [19]	Andhra Pradesh	India	Rectal swab samples from healthy dogs	2/92 (2.17%)	Combination disc method	ESBL producing *Pseudomonas* species
Sharif et al., 2017 [20]	Andhra Pradesh	India	Rectal swab samples from diarrheic dogs	5/44 (11.36%)	Combination disc method	ESBL producing *Pseudomonas* spp.
Shrivastav et al., 2016 [41]	Madhya Pradesh	India	Cecal swab samples in healthy broilers	135/400 (33.75%)	Combined disc diffusion test, DDST, Enz MIC strip	ESBL producing *E. coli*
Tewari et al., 2018 [27]	Assam	India	Fecal samples of livestock	10/48 (20.83%)	PCR-based detection	ESBL producing E. coli
Tewari et al., 2019 [30]	Meghalaya and Assam	India	Fecal samples of livestock	24/32 (75%)	PCR-based detection	ESBL producing E. coli

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study was conducted, total sample size, strains detected ESBL positive, and method used for confirmation of ESBL producing pathogen. Any inconsistency in data collection was rectified by re-checking the articles for accuracy.

Quality assessment

Since it is a prevalence study, use of Newcastle-Ottawa scale is not recommend. However, quality assessment of the study was done on fixed rating scale. This scale includes evaluation of study selection, comparability and outcome, with each section having maximum number of stars as 5, 3 and 2 respectively. Hence, the overall quality assessment has a maximum score of 10 and minimum score for inclusion is 3 stars. Table 2 shows the risk of bias assessment for the studies included in quantitative synthesis.

Statistical analysis

Meta-analysis for the prevalence of ESBL producing pathogens among animal samples were conducted using the R Open source scripting software (version 3.4.3, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/) [10]. The inbuilt packages used for analysis were Metafor and Meta R packages.

In the analysis, both random effect and fixed effect model were used to calculate the pooled prevalence of ESBL and I^2 statistic (to measure inconsistency). The τ^2 statistic was also calculated to measure the heterogeneity. Further, sub-group analysis was performed to reduce heterogeneity. In the present study, the data was stratified based on: year-wise (2013–2019) zone-wise (North, East, West, South and Central zones) and species-wise (*E. coli*, *Pseudomonas* spp., and *K. pneumoniae*).

Table 2. Meta-analysis of ESBL prevalence in animals from India.

Sl no.	Parameters	Period	Number of Articles	Number of Studies	Total Events	Pooled Prevalence(With 95% Confidence Interval)	I^2 Value (%)	τ^2 Value	p Value
1.	ESBL prevalence in Animals	2013–2019	17	27	6020	10 (7–15)	94	0.8090	p<0.01
				Year-Wise					
1.	Year 2013	2013	2	2	238	12 (2–62%)	95	1.7709	p<0.01
2.	Year 2014	2014	2	5	203	5 (2–9%)	0	0	p = 0.81
3.	Year 2015	2015	5	6	950	8 (3–18%)	91	1.1334	p<0.01
4.	Year 2016	2016	3	7	916	8 (4–16%)	93	0.6677	p<0.01
5.	Year 2017	2017	5	7	2279	12 (6–22%)	91	1.0612	p<0.01
6.	Year 2018	2018	3	3	1213	13(3–55%)	95	1.6353	p<0.01
7.	Year 2019	2019	3	3	221	33(13–81)	97	0.6148	p<0.01
				Zone-Wise					
1.	North Zone	2013–2017	2	2	2397	26 (19–36%)	96	0.0711	p<0.01
2.	East Zone	2013–2017	10	14	1978	11 (6–18%)	95	0.9394	p<0.01
3.	West Zone	-	0	0	-	-	-	-	-
4.	South Zone	2014–2017	2	5	226	6 (3–11%)	36	0.2815	p = 0.20
5.	Central Zone	2016–2017	3	6	806	8 (4–18%)	92	0.6864	p<0.01
				Species-Wise					
1.	*Escherichia coli*	2013–2017	11	17	4526	9 (6–15%)	92	0.8028	p<0.01
2.	*K. pneumoniae*	2013–2017	4	6	758	10 (6–19%)	85	0.3852	p<0.01
3.	*Pseudomonas* spp.	2017	1	2	136	5 (1–24%)	76	1.0345	p = 0.04

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Results

Distribution and characteristics of articles describing ESBLs in India

The electronic database searches returned 32 potential articles based on the keyword search. Review articles studying the ESBL prevalence in humans were excluded. A total of 23 articles were selected suitable for the study. The flowchart of systematic article selection is shown in Fig 1. All the articles included in the study described the prevalence of ESBL producing pathogens isolated from animals/animal samples from India. The maximum number of studies on this subject were found in the eastern zone followed by central, south and north zone. No studies were found from western zone of India. In total, 20 studies were on ESBLs produced by *Escherichia coli*, 6 on ESBLs produced by *K. pneumoniae* and 2 on ESBLs produced from *Pseudomonas* spp. The animal samples studied in the articles mainly included meat samples, milk samples, rectal swabs, cecal swabs and cloacal swabs from poultry birds, sheep, pig and cattle.

Pooled prevalence of ESBLs in animal samples

The meta-analysis revealed the overall pooled prevalence of ESBL in animals to be 9% (95% CI: 6–13%; $\tau^2 = 0.6654$; $P < 0.01^{**}$). The prevalence estimates of ESBL producing pathogens in India is depicted in the forest plot in Fig 2, which also displays the author, year, samples, events and total samples [11–20]. In order to reduce the heterogeneity, the studies on ESBL producing isolates were categorized by Year, Zone and Species-wise (Table 3). The pooled prevalence

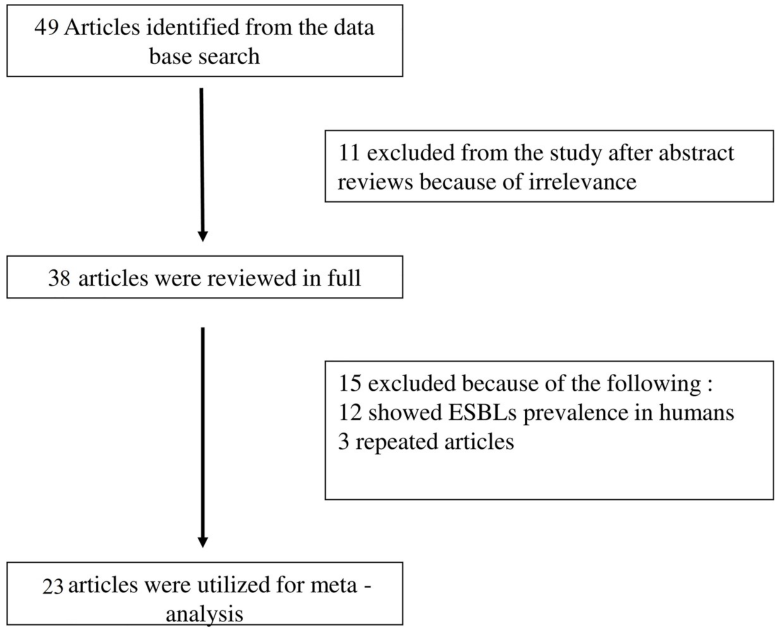

Fig 1. The flow diagram of study selection process.

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of ESBL producing pathogens in animals were 12, 5, 8, 8, 12, 13 and 33% for the years 2013, 2014, 2015, 2016, 2017, 2018 and 2019 respectively, as depicted in the forest plot [20–30] in (Fig 3A–3G). The zone-wise prevalence percentage of ESBLs were 26, 11, 6 and 8% for the north, east, south and central zones are shown in (Fig 4A–4D). The species-wise prevalence of ESBLs were found to be 9, 10 and 5% for *E.coli*, *K. pneumoniae* and *Pseudomonas spp*. respectively. Figs 5–7 explains the forest plot of species-wise Meta-analysis.

Discussion

Our study revealed that, the ESBL producing clinical isolates in India may not be very high, nonetheless it is significant. These drug-resistant pathogens are a serious concern worldwide

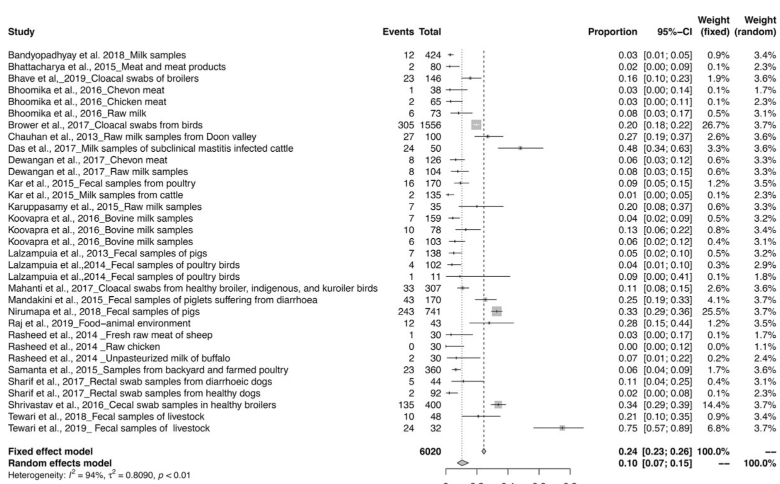

Fig 2. Forest plot of ESBL prevalence in India from 2013–2019.

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Table 3. Risk of bias assessment for studies included in the quantitative synthesis.

Author and year of publication	Selection		Comparability	Outcome	Overall Quality Assessment score
	Representativeness of the sample	Ascertainment of exposure		Assessment of outcome	
Bandyopadhyay et al. 2018 [25]	*Truly representative bovine milk samples	**ESBL production confirmed by PCR	Study did not control for other factors	*Independent blind assessment	3
Bhattacharya et al., 2015 [20]	*Truly representative Meat and meat products with antibiotic resistance	*ESBL production diagnosed by Combined Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Bhave et al., 2019 [28]	*Truly representative Cloacal swabs from broiler	*ESBL production diagnosed by Combined Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	4
Bhoomika et al., 2016 [10]	*Truly representative of chicken meat samples with antibiotic resistance	**Chicken meat samples diagnosed with clinical isolates producing ESBL confirmed by Multiplex PCR	Study did not control for other factors	*Independent blind assessment	4
Bhoomika et al., 2016 [10]	*Truly representative of chevon meat samples with antibiotic resistance	**Chevon meat samples diagnosed with clinical isolates producing ESBL confirmed by Multiplex PCR	Study did not control for other factors	*Independent blind assessment	4
Bhoomika et al., 2016 [10]	*Truly representative of raw milk samples with antibiotic resistance	**Raw milk samples diagnosed with clinical isolates producing ESBL confirmed by Multiplex PCR	Study did not control for other factors	*Independent blind assessment	4
Brower et al., 2017 [11]	*Truly representative Cloacal swabs from birds with antibiotic resistance	*ESBL production diagnosed by Combination disk method and VITEK 2	Study did not control for other factors	*Independent blind assessment	3
Chauhan et al., 2013 [12]	*Truly representative Raw milk samples from Doon valley with antibiotic resistance	*ESBL production diagnosed by Double disc diffusion method	Study did not control for other factors	*Independent blind assessment	3
Das et al., 2017 [14]	*Truly representative of sub-clinical mastic milk samples with antibiotic resistance	**Sub-clinical mastic milk samples diagnosed with clinical isolates producing ESBL confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4
Dewangan et al., 2017 [15]	*Truly representative Chevon meat with antibiotic resistance	*ESBL production diagnosed by Phenotypic detection of ESBL	Study did not control for other factors	*Independent blind assessment	3
Dewangan et al., 2017 [15]	*Truly representative Raw milk samples with antibiotic resistance	*ESBL production diagnosed by Phenotypic detection of ESBL	Study did not control for other factors	*Independent blind assessment	3
Kar et al., 2015 [21]	*Truly representative Fecal samples from poultry with antibiotic resistance	*ESBL production diagnosed by Combination disc method and ESBL E-test	Study did not control for other factors	*Independent blind assessment	3
Kar et al., 2015 [21]	*Truly representative Milk samples from cattle with antibiotic resistance	*ESBL production diagnosed by Combination disc method and ESBL E-test	Study did not control for other factors	*Independent blind assessment	3
Karuppasamy et al., 2015 [22]	*Truly representative Raw milk samples with antibiotic resistance	*ESBL production diagnosed by Kirby Bauer disc diffusion test	Study did not control for other factors	*Independent blind assessment	3
Koovapra et al.,2016 [33]	*Truly representative Bovine milk samples with antibiotic resistance	ESBL production diagnosed by Combination disc diffusion test and ESBL Etest	Study did not control for other factors	*Independent blind assessment	3
Lalzampuia et al., 2013 [16]	*Truly representative Fecal samples of pigs with antibiotic resistance	**Pigs with history of diarrhea diagnosed with clinical isolates producing ESBL confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4
Lalzampuia et al., 2014 [17]	*Truly representative Fecal samples of poultry birds with antibiotic resistance	**Poultry birds with history of diarrhea diagnosed with clinical isolates producing ESBL confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4

(*Continued*)

Table 3. (Continued)

Author and year of publication	Selection		Comparability	Outcome	Overall Quality Assessment score
	Representativeness of the sample	Ascertainment of exposure		Assessment of outcome	
Lalzampuia et al., 2014 [17]	*Truly representative Fecal samples of poultry birds with antibiotic resistance	**Poultry birds with history of diarrhea diagnosed with clinical isolates producing ESBL confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4
Mahanti et al., 2017 [13]	*Truly representative Cloacal swabs from healthy broiler, indigenous, and kuroiler birds with antibiotic resistance	**ESBL production confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4
Mandakini et al., 2015 [23]	*Truly representative Fecal samples of piglets suffering from diarrhea with antibiotic resistance	*ESBL production diagnosed by Double disc synergy test	Study did not control for other factors	*Independent blind assessment	3
Nirumapa et al., 2018 [26]	*Truly representative Fecal samples of pigs	** ESBL production diagnosed by Double disc diffusion method and Hi-comb MIC test strip	Study did not control for other factors	*Independent blind assessment	3
Raj et al., 2019 [29]	* Truly representative Food-animal environment	**ESBL production diagnosed by PCR	Study did not control for other factors	*Independent blind assessment	3
Rasheed et al., 2014 [18]	*Truly representative Unpasteurized milk of buffalo with antibiotic resistance	*ESBL production diagnosed by Phenotypic Confirmatory Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Rasheed et al., 2014 [18]	*Truly representative Raw chicken with antibiotic resistance	*ESBL production diagnosed by Phenotypic Confirmatory Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Rasheed et al., 2014 [18]	*Truly representative Fresh raw meat of sheep with antibiotic resistance	*ESBL production diagnosed by Phenotypic Confirmatory Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Samanta et al., 2015 [24]	*Truly representative Samples from backyard and farmed poultry with antibiotic resistance	**ESBL production confirmed by PCR	Study did not control for other factors	*Independent blind assessment	4
Sharif et al., 2017 [19]	*Truly representative Rectal swab samples from healthy dogs with antibiotic resistance	*ESBL production diagnosed by Combined Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Sharif et al., 2017 [19]	*Truly representative Rectal swab samples from diarrheic dogs with antibiotic resistance	*ESBL production diagnosed by Combined Disc Diffusion Test	Study did not control for other factors	*Independent blind assessment	3
Shrivastav et al., 2016 [35]	*Truly representative Cecal swab samples in healthy broilers with antibiotic resistance	*ESBL production diagnosed by CDDT, DDST and Enz MIC strip in Healthy broilers	Study did not control for other factors	*Independent blind assessment	3
Tewari et al., 2018 [27]	*Truly representative Fecal samples of livestock	**ESBL production confirmed by PCR	Study did not control for other factors	*Independent blind assessment	3
Tewari et al., 2019 [30]	*Truly representative Fecal samples of livestock	**ESBL production confirmed by PCR	Study did not control for other factors	*Independent blind assessment	3

PCR, Polymerase chain Reaction; CDDT, Combined disc diffusion test; DDST, Double disc synergy test, Enz MIC strip, Enz Minimum Inhibitory Concentration strip; E test, Epsilometer test.

(*)Stars represent the number of points awarded for the category;

* = 1,

** = 2.

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as they are associated with increase in morbidity and mortality rate due to infections they cause [31]. Extended-Spectrum Beta-Lactamases are produced by species of bacteria in order

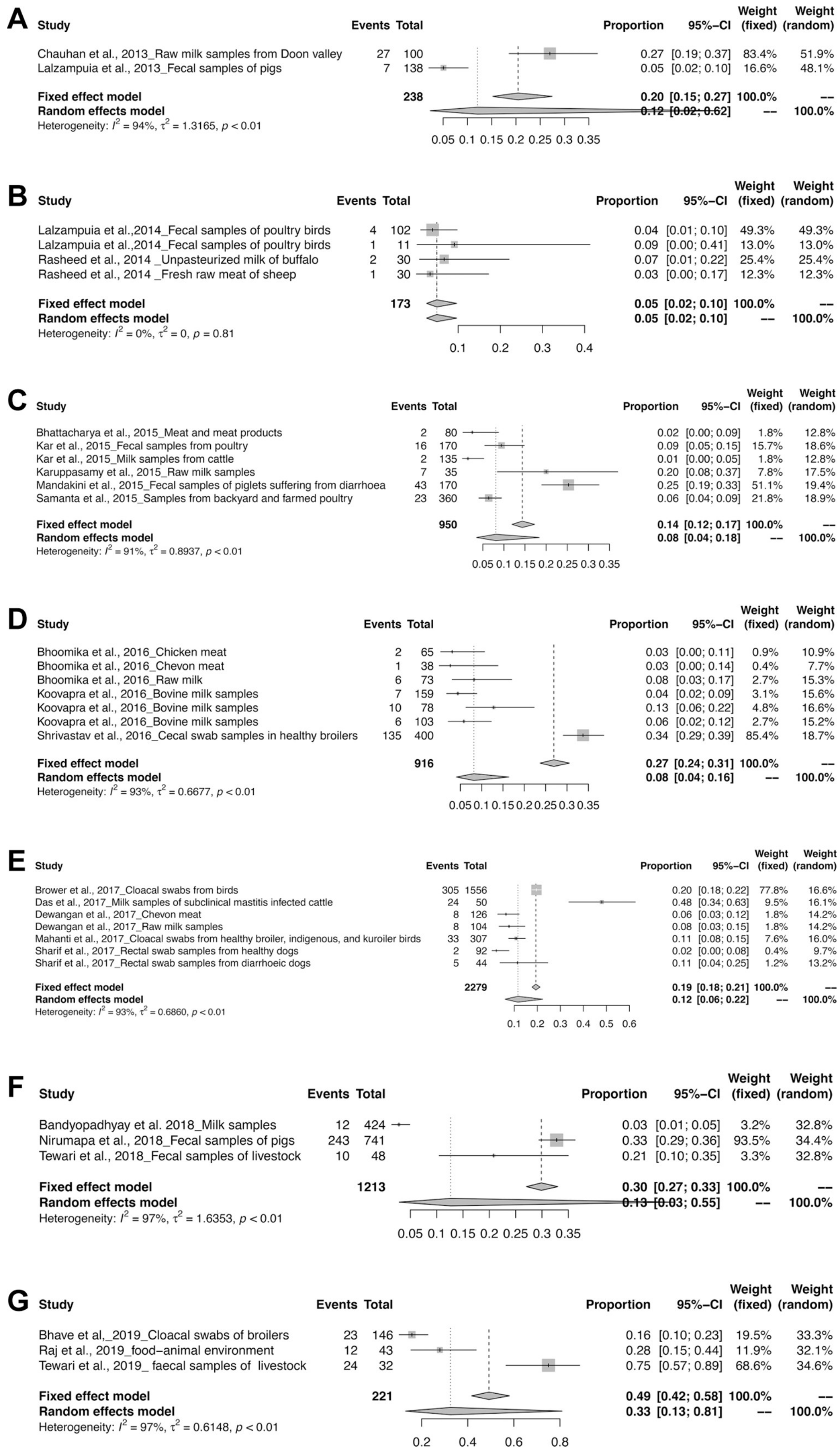
A
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Chauhan et al., 2013_Raw milk samples from Doon valley 27 100 0.27 [0.19; 0.37] 83.4% 51.9%
Lalzampuia et al., 2013_Fecal samples of pigs 7 138 0.05 [0.02; 0.10] 16.6% 48.1%
Fixed effect model 238 0.20 [0.15; 0.27] 100.0% --
Random effects model 0.12 [0.02; 0.62] -- 100.0%
Heterogeneity: $I^2 = 94\%$, $\tau^2 = 1.3165$, $p < 0.01$
0.05 0.1 0.15 0.2 0.25 0.3 0.35
B
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Lalzampuia et al.,2014_Fecal samples of poultry birds 4 102 0.04 [0.01; 0.10] 49.3% 49.3%
Lalzampuia et al.,2014_Fecal samples of poultry birds 1 11 0.09 [0.00; 0.41] 13.0% 13.0%
Rasheed et al., 2014 _Unpasteurized milk of buffalo 2 30 0.07 [0.01; 0.22] 25.4% 25.4%
Rasheed et al., 2014 _Fresh raw meat of sheep 1 30 0.03 [0.00; 0.17] 12.3% 12.3%
Fixed effect model 173 0.05 [0.02; 0.10] 100.0% --
Random effects model 0.05 [0.02; 0.10] -- 100.0%
Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, $p = 0.81$
0.1 0.2 0.3 0.4
C
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Bhattacharya et al., 2015_Meat and meat products 2 80 0.02 [0.00; 0.09] 1.8% 12.8%
Kar et al., 2015_Fecal samples from poultry 16 170 0.09 [0.05; 0.15] 15.7% 18.6%
Kar et al., 2015_Milk samples from cattle 2 135 0.01 [0.00; 0.05] 1.8% 12.8%
Karuppasamy et al., 2015_Raw milk samples 7 35 0.20 [0.08; 0.37] 7.8% 17.5%
Mandakini et al., 2015_Fecal samples of piglets suffering from diarrhoea 43 170 0.25 [0.19; 0.33] 51.1% 19.4%
Samanta et al., 2015_Samples from backyard and farmed poultry 23 360 0.06 [0.04; 0.09] 21.8% 18.9%
Fixed effect model 950 0.14 [0.12; 0.17] 100.0% --
Random effects model 0.08 [0.04; 0.18] -- 100.0%
Heterogeneity: $I^2 = 91\%$, $\tau^2 = 0.8937$, $p < 0.01$
0.05 0.1 0.15 0.2 0.25 0.3 0.35
D
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Bhoomika et al., 2016_Chicken meat 2 65 0.03 [0.00; 0.11] 0.9% 10.9%
Bhoomika et al., 2016_Chevon meat 1 38 0.03 [0.00; 0.14] 0.4% 7.7%
Bhoomika et al., 2016_Raw milk 6 73 0.08 [0.03; 0.17] 2.7% 15.3%
Koovapra et al., 2016_Bovine milk samples 7 159 0.04 [0.02; 0.09] 3.1% 15.6%
Koovapra et al., 2016_Bovine milk samples 10 78 0.13 [0.06; 0.22] 4.8% 16.6%
Koovapra et al., 2016_Bovine milk samples 6 103 0.06 [0.02; 0.12] 2.7% 15.2%
Shrivastav et al., 2016_Cecal swab samples in healthy broilers 135 400 0.34 [0.29; 0.39] 85.4% 18.7%
Fixed effect model 916 0.27 [0.24; 0.31] 100.0% --
Random effects model 0.08 [0.04; 0.16] -- 100.0%
Heterogeneity: $I^2 = 93\%$, $\tau^2 = 0.6677$, $p < 0.01$
0.05 0.1 0.15 0.2 0.25 0.3 0.35
E
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Brower et al., 2017_Cloacal swabs from birds 305 1556 0.20 [0.18; 0.22] 77.8% 16.6%
Das et al., 2017_Milk samples of subclinical mastitis infected cattle 24 50 0.48 [0.34; 0.63] 9.5% 16.1%
Dewangan et al., 2017_Chevon meat 8 126 0.06 [0.03; 0.12] 1.8% 14.2%
Dewangan et al., 2017_Raw milk samples 8 104 0.08 [0.03; 0.15] 1.8% 14.2%
Mahanti et al., 2017_Cloacal swabs from healthy broiler, indigenous, and kuroiler birds 33 307 0.11 [0.08; 0.15] 7.6% 16.0%
Sharif et al., 2017_Rectal swab samples from healthy dogs 2 92 0.02 [0.00; 0.08] 0.4% 9.7%
Sharif et al., 2017_Rectal swab samples from diarrhoeic dogs 5 44 0.11 [0.04; 0.25] 1.2% 13.2%
Fixed effect model 2279 0.19 [0.18; 0.21] 100.0% --
Random effects model 0.12 [0.06; 0.22] -- 100.0%
Heterogeneity: $I^2 = 93\%$, $\tau^2 = 0.6860$, $p < 0.01$
0.1 0.2 0.3 0.4 0.5 0.6
F
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Bandyopadhyay et al. 2018_Milk samples 12 424 0.03 [0.01; 0.05] 3.2% 32.8%
Nirumapa et al., 2018_Fecal samples of pigs 243 741 0.33 [0.29; 0.36] 93.5% 34.4%
Tewari et al., 2018_Fecal samples of livestock 10 48 0.21 [0.10; 0.35] 3.3% 32.8%
Fixed effect model 1213 0.30 [0.27; 0.33] 100.0% --
Random effects model 0.13 [0.03; 0.55] -- 100.0%
Heterogeneity: $I^2 = 97\%$, $\tau^2 = 1.6353$, $p < 0.01$
0.05 0.1 0.15 0.2 0.25 0.3 0.35
G
Study Events Total Proportion 95%−CI Weight (fixed) Weight (random)
Bhave et al,_2019_Cloacal swabs of broilers 23 146 0.16 [0.10; 0.23] 19.5% 33.3%
Raj et al., 2019_food−animal environment 12 43 0.28 [0.15; 0.44] 11.9% 32.1%
Tewari et al., 2019_ faecal samples of livestock 24 32 0.75 [0.57; 0.89] 68.6% 34.6%
Fixed effect model 221 0.49 [0.42; 0.58] 100.0% --
Random effects model 0.33 [0.13; 0.81] -- 100.0%
Heterogeneity: $I^2 = 97\%$, $\tau^2 = 0.6148$, $p < 0.01$
0.2 0.4 0.6 0.8

Fig 3. Forest plots of ESBL prevalence in (a) 2013; (b) 2014; (c) 2015; (d) 2016; (e) 2017; (f) 2018; and (g) 2019.

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to inactivate antibiotics, causing antibiotic resistance. Beta-lactamase seems to be the prime cause in multidrug resistant (MDR) *E. coli* strains. Early detection of *E. coli* that produce beta lactamase is necessary in order to prevent MDR *E. coli* from spreading [32]. Activity of ESBLs caused by different beta-lactamases resulted in resistant genes within the farm [33]. The strains that were isolated showed that a small portion of the resistant genes were present in one farm [4]. The steep rise in income and the growing population has driven an increase in demand for animal products in India. India is one of the top consumers of antibiotics worldwide, it accounts for about 3% of global consumption which is estimated to double by 2030. This could be due to the non-therapeutic use of antibiotics in cases of prophylaxis and growth promotion [34]. Currently, the usage of antibiotics is high in poultry, swine and cattle production as compared to that being used by the human population [35–36].

To address the concern of antimicrobial resistant bacteria, it is crucial to raise awareness of the problem by collecting data on antibiotic resistance from various countries and regions. The paucity of studies available from India affirms attention for future research. To our

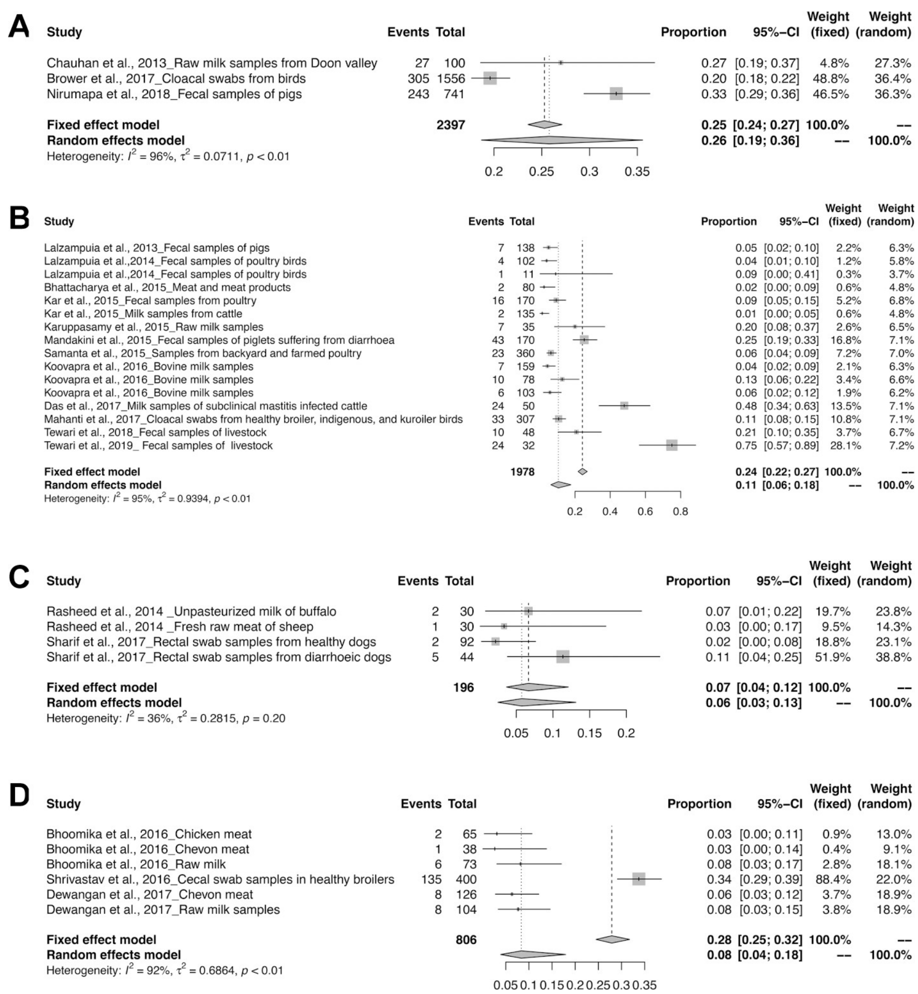

Fig 4. Forest plots of ESBL prevalence in (a) north-zone; (b) east-zone; (c) south-zone; and (d) central-zone.

https://doi.org/10.1371/journal.pone.0221771.g004

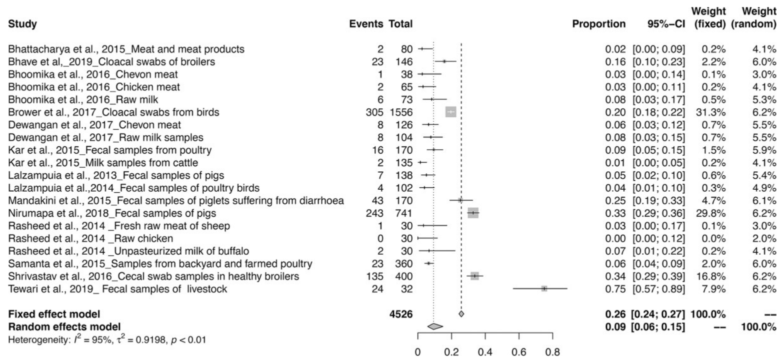

Fig 5. Forest plot of *E. coli* producing ESBL prevalence.

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knowledge, this is the first meta-analysis regarding the magnitude of the ESBL problem in Indian animal population. From the 23 articles chosen in the study, the overall pooled prevalence of ESBL producing isolates from the animal samples was found to be 10%. In Asia, high rates of ESBL producing *Enterobacteriaceae* are seen with variation in the prevalence and the genotype of the ESBL producing isolates over the large geographical area [30].

The prevalence of ESBL producing isolates were 12, 5, 8, 8, 12, 13 and 33% for the years 2013, 2014, 2015, 2016, 2017, 2018 and 2019 respectively, indicating an increase in the percent drug resistance since 2014 to 2019. The pooled prevalence of ESBL producing isolates was determined zone-wise and North zone showed a higher prevalence rate in comparison to other zones. Nonetheless, no studies on prevalence of ESBL producing isolates for animal samples from the Western zone of India are reported. Prevalence of species-wise classification was found to be 9, 10 and 5% for *E. coli*, *K. pneumoniae* and *Pseudomonas* spp. respectively, signifying that the ESBL producing *K. pneumoniae* is the most predominant ESBL producing isolate in India.

A study conducted in the intensive care units (ICUs) of an Indian hospital concluded that there is a need for constant surveillance to detect resistant bacterial strains, strict guidelines on antibiotic therapy, and effective infection control measures in order to reduce the spread of antibiotic resistant bacteria. The same study also revealed that there is a high number of ESBL producing *E. coli* in the ICUs of that hospital [31]. A study with pediatric and neonatal patients estimated the number of poor outcomes and indicated the association of blood stream infections (BSIs) with Extended-Spectrum Beta-Lactamase- producing *Enterobacteriaceae* (ESBL-PE). The results showed a high prevalence of BSIs due to ESBL-PE and increase in neonatal mortality [37–39]. A study from Germany demonstrated that direct transfer of ESBL-producing E. *coli* could occur between livestock and the farm workers who were in close contact with farm animals. The study also suggests an existence of epidemiological links between livestock and farm workers. A high prevalence of ESBL-producing *E. coli* in pig and cattle farms emphasizes the fact that livestock animals are a constant source for these potential human pathogens [33, 40–41].

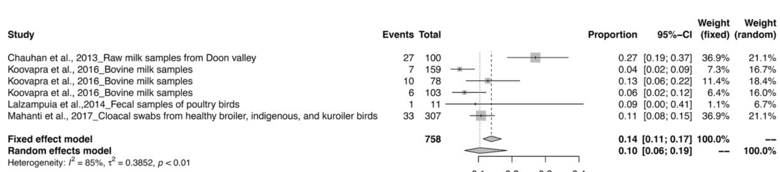

Fig 6. Forest plot of *K. pneumonia* producing ESBL prevalence.

https://doi.org/10.1371/journal.pone.0221771.g006

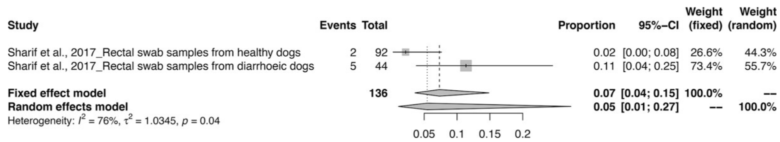

Fig 7. Forest plot of *Pseudomonas spp*. producing ESBL prevalence.

https://doi.org/10.1371/journal.pone.0221771.g007

Our research findings does have some minor limitations, which includes the lack of sufficient information on the prevalence of ESBL producers from different animal species. Upon advanced literature survey, we could find only a few articles that addressed the prevalence of ESBLs in animals.

Conclusion

India being a developing country, has the highest burden of bacterial infections. Hence, to combat this downfall, antibiotics are used widely and indiscriminately. The overuse, lack of awareness and non-therapeutic use of antibiotics is driving an increase in the antibiotic resistance among animals. This meta-analysis, indicated that the pooled prevalence of ESBLs for animals in India is not high, however, the overall prevalence remains significant at 10%. Additionally, only little information is currently available that addresses the prevalence of ESBLs in animals in India. The paucity of data on the clinical outcomes, magnitude and prevalence of the resistant ESBLs, calls for active surveillance which can help understand the epidemiology of ESBL burden in India. Furthermore, emphasis on awareness programs, personal and environmental hygiene should be implemented to stop and manage the spread of ESBLs to the animals and environment. Further studies are needed to better understand the complexity of the AMR problem in animal and human population.

Supporting information

S1 File. PRISMA checklist.
(DOC)

S2 File. Listed references for underlying data.
(DOCX)

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Author Contributions

Conceptualization: Sharanagouda S. Patil, Raghavendra G. Amachawadi.

Data curation: Suresh P. Kuralayanapalya, Sudhakar Hamsapriya, Rajamani Shinduja, Raghavendra G. Amachawadi.

Formal analysis: Suresh P. Kuralayanapalya, Sudhakar Hamsapriya.

Investigation: Raghavendra G. Amachawadi.

Methodology: Suresh P. Kuralayanapalya.

Supervision: Raghavendra G. Amachawadi.

Visualization: Sharanagouda S. Patil.

Writing – original draft: Suresh P. Kuralayanapalya, Sudhakar Hamsapriya, Rajamani Shinduja, Raghavendra G. Amachawadi.

Writing – review & editing: Suresh P. Kuralayanapalya, Parimal Roy, Raghavendra G. Amachawadi.

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