


Bacterial pathogens identified from houseflies in different human and animal settings: A systematic review and meta-analysis

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Abstract

Housefly (*Musca domestica*) is an excellent candidate for the distribution of susceptible and resistant bacterial strains that potentially threaten public health. To date, there is a paucity of information on the global distribution of pathogenic bacteria of medical and veterinary importance from diverse environmental settings. Therefore, this study was undertaken to conduct a systemic review and meta-analysis to estimate occurrence of various bacterial species of medical and veterinary importance harboured by houseflies around the world. Published articles from 1980 to 2020 were retrieved from electronic databases and assessed for eligibility according to Preferred Reporting Items for Systemic Reviews and Meta-Analysis guidelines. Seventy-eight studies were included in the review with only 44 studies being eligible for meta-analysis. Results indicated that eligible studies used in this review were from four continents, i.e., Asia (47.4%) America (23.1%), Africa (20.5%) and Europe (8.9%). The majority of the studies (56.4%) used the culture method for the identification of bacterial pathogens, while 30.7% used both culture and PCR techniques. For meta-analysis, we focused on five pathogenic bacterial species including *Escherichia coli*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. High heterogeneity was found among studies investigating different pathogens including *E. coli* ($Q = 10,739.55$; $I^2 = 99.60$; $Q-p < 0.0001$), *E. faecium* ($Q = 317.61$; $I^2 = 86.46$; $Q-p < 0.0001$), *K. pneumoniae* ($Q = 1,576.61$; $I^2 = 97.27$; $Q-p < 0.0001$), *S. aureus* ($Q = 2,439.12$; $I^2 = 98.24$; $Q-p < 0.0001$) and *P. aeruginosa* ($Q = 1,283.0$; $I^2 = 96.65$; $Q-p < 0.0001$). Furthermore, it was observed that houseflies carried a considerable number of susceptible and antibiotic-resistant bacterial strains that pose considerable threats to public health. Findings from this study have provided more insight on the vectoral potential of houseflies in the transmission of significant bacterial pathogens from different regions across the world. Further investigation is required to quantify the bacterial contamination and dissemination by houseflies.

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KEYWORDS

animals, bacteria, houseflies, humans

Impacts

- Houseflies are in abundance in different human and animal settings due to their behavioural characteristics.
- Houseflies carry a considerable number of susceptible and antibiotic-resistant bacteria, including multiple-antibiotic resistant strains that could pose severe threat to public health.
- Majority of studies have used culture methods for detection of bacteria from houseflies, but recently there is an increase in use of DNA-based techniques.

1 | INTRODUCTION

Houseflies exist as major pests of humans, poultry and livestock surroundings and facilities where they transmit vector-borne infections globally (Ahmed et al., 2013; Akter et al., 2020; Iqbal et al., 2014). Based on World Health Organization (WHO) records, the major vector-borne infections account for almost 17% of communicable diseases that occurs per annum worldwide, with high incidence in tropical and subtropical regions (WHO, 2017).

Houseflies are common and occur in high abundance in close association with human activities. These insects are attracted to human and animal waste for feeding and reproduction (Awache & Forouk, 2016). Human (i.e., hospitals, food markets and restaurants) and animal environments (i.e., farms, feedlots and slaughterhouses) are the major points of focus and sampled areas for houseflies. Owing to their diverse habitat preference, indiscriminate movement, the ability to fly long distance and attraction to both decaying organic materials, houseflies greatly amplify the risk of human exposure to variety of pathogens including bacteria (Barreiro et al., 2013; Gupta et al., 2012).

The reproduction habits and feeding mechanisms of houseflies involve microbe-rich substrates (Park et al., 2019). Thus, houseflies harbour bacteria in both immature and mature life stages (Zurek et al., 2000) and are widely considered a significant source of bacterial diversity (Wei et al., 2013). The bacterial species found to colonize houseflies include *Salmonella* spp., *Yersinia enterocolitica*, *Edwardsiella tarda*, *Shigella sonnei*, *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Rahuma et al., 2005), to name a few. The characteristics of bacterial genera carried by houseflies are dependent on the sampled area (Khamesipour et al., 2018). Thus, houseflies can transport a diverse and active microbial community from different reservoirs (Barreiro et al., 2013; Gupta et al., 2012). Therefore, these pathogens may cause serious infections and diseases to susceptible humans and animals (Ahmed et al., 2013; Malik et al., 2007).

The risk of houseflies to disseminate the antibiotic resistant bacterial strains from hospital settings, livestock and poultry farms to public residents is of great concern (Zhang et al., 2018). According to Zhang et al. (2018), houseflies sampled from these areas with extensive usage of antibiotics are commonly colonized

by antibiotic-resistant bacteria including bacterial species that are multidrug resistant. An increasing frequency of antibiotic resistance has been reported from all over the world (Davari et al., 2010). Thus, the emergence of bacteria that is resistant to critically important antimicrobials is of a major concern in human and veterinary medicine (Solà-Ginés et al., 2015).

Houseflies colonized with these bacterial species may also be associated with the dissemination of antibiotic resistant genes or exposure to virulent bacterial strains within the same environment (Bouamama et al., 2010; Zhang et al., 2018). Transmission takes place mostly when the houseflies come in contact with animals, people and their food (WHO, 1997). Thus, houseflies contribute to the mechanical transmission of foodborne pathogens and other serious diseases that can affect humans and animals. These diseases include anthrax, ophthalmia, typhoid fever, tuberculosis, cholera and infantile diarrhoea (Khamesipour et al., 2018; WHO, 1997).

The current systematic review and meta-analysis was carried out to determine the pathogenic bacteria isolated from houseflies by various studies conducted around the world, and how frequent these studies report on the antibiotic susceptibility profiles and resistant genes in common bacterial pathogens. This was performed to generate evidence and knowledge necessary to help formulate preventative measures against infectious bacteria harboured by houseflies.

2 | MATERIALS AND METHODS**2.1 | Systematic search strategy and selection criteria of the journal articles**

This review was done following the recommended methodology as outlined by the preferred reporting items for systematic reviews (PRISMA) as shown in Figure 1 (Moher et al., 2015). The literature search was performed to identify journal articles published from 1980 to 2020 reporting on housefly or *Musca domestica* and bacteria. The phrase '*Musca domestica*' was searched following the combination of free text and thesaurus terms in different variations: houseflies and bacterial pathogens. These keywords were used to retrieve studies from electronic databases including

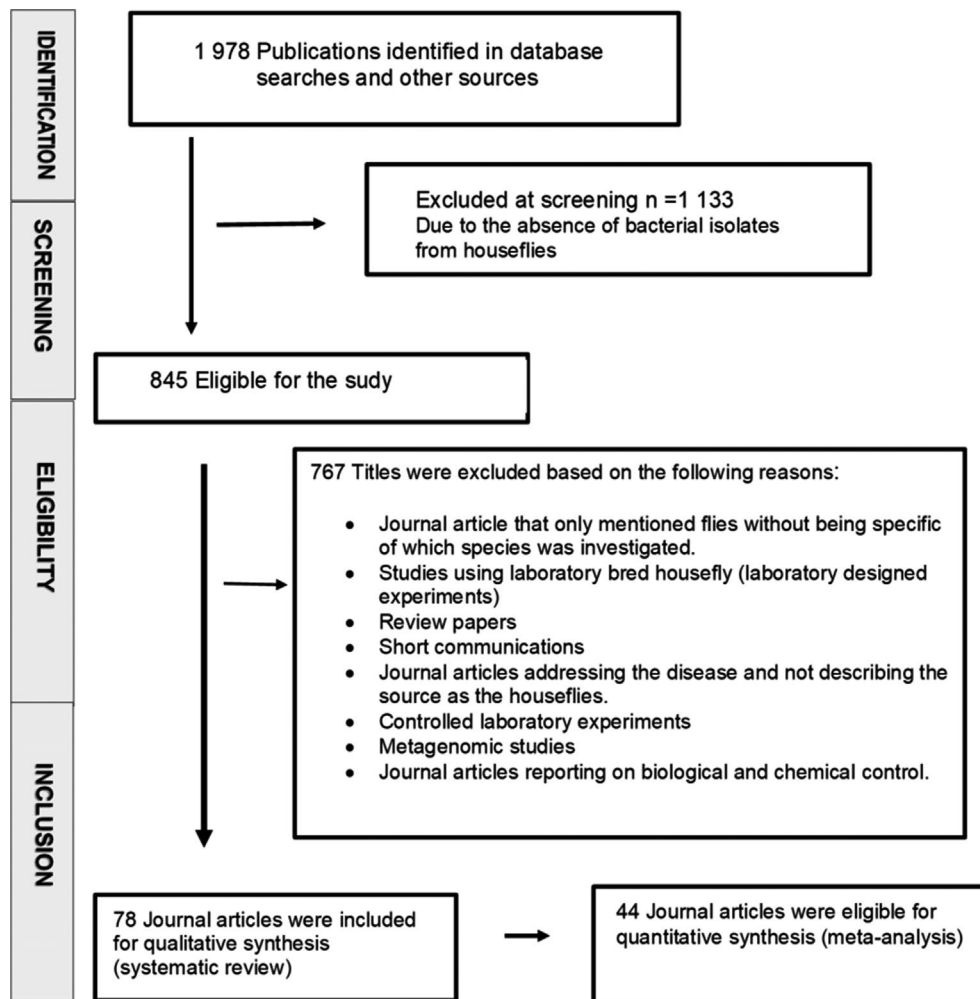


FIGURE 1 Selection process of eligible studies following PRISMA guidelines

PubMed (<https://pubmed.ncbi.nlm.nih.gov/>, from 01/09/2019 to 08/06/2020); Science Direct (<https://www.sciencedirect.com/> from 06/09/2019 to 08/06/2020); and Google scholar (<https://scholar.google.com/> from 11/09/2019 to 08/06/2020) with the last search done on the 8th of June 2020. The systematic search was accomplished following the combination of free text and wordlist terms in diverse distinctions: 'Houseflies' AND/OR, '*Musca domestica*', 'Bacterial pathogens' AND/OR 'Bacterial isolation'. Thereafter, titles and abstract were scanned and potential journal articles were reviewed and downloaded. The reference list from the potential searched published journal articles was also screened for journal articles relating to this review.

2.2 | Inclusion and exclusion criteria for systematic review

The search was limited to only to the English language and conducted from 1 September 2019 to 8 June 2020. The abstracts were assessed and the scientific papers that met a preceding inclusion criterion were selected. Preliminary screening of titles

and abstracts was carried out for eligibility and relevance to this systematic review and meta-analysis according to the inclusion and exclusion criteria. The published journal articles selected in this study were read for data extraction and screened further by a detailed review of the full text. The articles had to fulfil the following criteria to be included: (a) original research articles from around the world, (b) the availability of the full texts, and (c) journal articles published in English language (d) conducted between 1 September 2019 and 8 June 2020; (e) study design was cross-sectional/prevalence study; (f) the laboratory examination methods used was clearly stated; (g) the geographical location of the study was clearly stated; (h) the species of *M. domestica* was clearly stated; (i) the number of *M. domestica* with bacterial positive cases and sample size were provided; (j) the study screened for all bacterial species. Articles were excluded if they were published only in abstract form, or if they were editorials, letters, comments or reviews. Furthermore, the excluded articles were those reporting pathogens isolated from flies in general without specifying the fly species, studies using laboratory-reared *M. domestica* (laboratory designed experiments), journal articles addressing the disease and not describing the source as *M. domestica*, controlled laboratory

TABLE 1 Meta-analysis of eligible studies reporting on selected pathogenic bacteria isolated from houseflies in different regions

Study reference	Region	Method of diagnosis utilized	Sample size	<i>E. coli</i> no. of positives: (prevalence)	<i>E. faecium</i> no. of positives: (Prevalence)	<i>S. aureus</i> no. of positives: (Prevalence)	<i>K. pneumoniae</i> no. of positives: (Prevalence)	<i>P. aeruginosa</i> no. of positives: (Prevalence)
Fotedar, Banerjee, Shrinivas, and Verma (1992)	India	Culture	113	22: (19.5%)	—	24: (21.2%)	—	5: (4.4%)
Bouamama et al. (2010)	Morocco	Culture	600	26: (4.3%)	—	8: (1.3%)	—	—
Fotedar, Banarjee, Samantray, and Shrinivas (1992)	India	Culture	240	—	—	—	45: (18.8%)	—
Hemmatinezhad et al. (2015)	Iran	Culture PCR	600	—	—	—	—	53: (8.8%)
Macovei and Zurek (2006)	USA	PCR	268	—	14: (5.2%)	—	—	—
Macovei et al. (2008)	USA	Culture PCR	75	—	3: (4%)	—	—	—
Solà-Ginés et al. (2015)	Spain	Culture	615	41: (6.7%)	—	—	—	—
Mohammed et al. (2016)	Egypt	Culture	22	6: (27.3%)	—	2: (9.1%)	—	—
Nmorsi et al. (2007)	Nigeria	Culture	737	194: (26.3%)	—	—	—	—
Ranjbar et al. (2016)	Iran	Culture PCR	600	—	—	—	68: (11.3%)	—
Sobur et al. (2019)	Bangladesh	Culture PCR	300	40: (13.3%)	—	—	—	—
Songe et al. (2016)	Zambia	Culture PCR	418	307: (73.4%)	—	—	—	—
Usui et al. (2013)	Japan	Culture PCR	91	41: (45.1%)	—	—	—	—
Usui et al. (2015)	Japan	Culture PCR	74	13: (17.6%)	—	—	—	—
Yosboonruang et al. (2019)	Thailand	Culture PCR	60	60: (100%)	—	—	—	—
Al-Yousef. (2014)	Saudi Arabia	Culture	200	85: (42.5%)	—	21: (10.5%)	—	22: (11%)
Baker et al. (2018)	Iraq	Culture	150	36: (24%)	—	25: (1.3%)	11: (7.3%)	—
Burrus et al. (2017)	USA	Culture PCR	35	27: (77.1%)	—	—	—	—
Doud et al. (2014)	USA	PCR	276	—	60: (21.7%)	—	—	—
Manandhar and Gokhale (2017)	Nepal	Culture PCR	100	8: (8%)	—	4: (4%)	—	—
Nazari et al. (2017)	Iran	Culture	300	46: (15.3%)	—	25: (8.3%)	—	—
Neupane et al. (2019)	USA	Culture PCR	60	—	—	—	—	—

(Continues)

TABLE 1 (Continued)

Study reference	Region	Method of diagnosis utilized	Sample size	<i>E. coli</i> no. of positives: (prevalence)	<i>E. faecium</i> no. of positives: (Prevalence)	<i>S. aureus</i> no. of positives: (Prevalence)	<i>K. pneumoniae</i> no. of positives: (Prevalence)	<i>P. aeruginosano.</i> of positives: (Prevalence)
Pohlentz et al. (2018)	USA	Culture	224	205: (91.1%)	—	—	—	—
Puri-Giri et al. (2017)	USA	Culture PCR	463	159: (34.3%)	—	—	—	—
Rosef and Kapperud (1983)	Norway	Culture	608	—	—	—	—	—
Vaziriannzadeh et al. (2008)	Iran	Culture	230	230: (100%)	—	169: (73.5%)	—	—
Tsangaan et al. (2015)	Japan	PCR	267	114: (42.7%)	—	—	—	—
Szalanski et al. (2004)	USA	Culture PCR	2,486	160: (6.4%)	—	—	—	—
Rahuma et al. (2005)	Libya	Culture	150	58: (38.7%)	—	—	—	—
Yalli et al. (2017)	Nigeria	Culture	150	11: (7.3%)	—	12: (8%)	—	—
Nwankwo et al. (2020)	Nigeria	Culture	150	35: (23.3%)	—	23: (15.3%)	—	9: (6%)
Davari et al. (2010)	Iran	Culture	908	45: (4.95%)	—	—	—	77: (8.5%)
Awache and Forouk (2016)	Nigeria	Culture	1,059	177: (16.7%)	—	—	113: (10.7%)	—
Akter et al. (2020)	Bangladesh	Culture PCR	140	72: (51.4%)	—	110: (78.6%)	—	—
Mawak and Olukose (2006)	Nigeria	Culture	200	30: (15%)	—	—	—	—
Chaiwong et al. (2014)	Thailand	culture	1,349	94: (6.96%)	—	13: (0.96%)	—	97: (7.2%)
Ghalehnoo (2015)	Iran	Culture	450	—	—	87: (19.3%)	—	—
Kababian et al. (2020)	Iran	Culture	160	59: (36.9%)	—	27: (16.9%)	—	33: (20.6%)
Moriya et al. (1999)	Japan	Culture PCR	89	10: (11.2%)	—	—	—	—
Odentoyin et al. (2020)	Nigeria	Culture	25	25: (100%)	—	11: (44%)	17: (68%)	3: (12%)
Akter et al. (2017)	Bangladesh	Culture	15	15: (100%)	—	—	—	—
Brits et al. (2016)	South Africa	Culture PCR	10	—	—	—	—	—
Alam and Zurek (2004)	USA	Culture PCR	3,440	75: (2.2%)	—	—	—	—
Choo et al. (2011)	Malaysia	Culture	60	—	—	—	—	—

Abbreviations: PCR, polymerase chain reaction; USA, United States of America.

experiments and metagenomic studies (Figure 1). No attempt was made to contact the authors of the original manuscripts for any additional information or retrieval of unpublished studies. The data was retrieved by one investigator and then confirmed by another investigator.

2.3 | Data extraction and meta-analysis

Key characteristics of each included article were summarized in a spreadsheet (Microsoft Excel®), with the following variables: author name, country; sites of sample collection, diagnostic technique and bacterial isolates. Several bacteria species were reported from all the included studies. For the sampling locations, closely related sampled places were grouped together. Only journal articles which were definite with the species of bacteria were included for meta-analysis. Five selected pathogenic bacteria, namely, *E. coli*, *E. faecium*, *Klebsiella pneumoniae*, *P. aeruginosa* and *S. aureus* were focused on. The prevalence for the subgroups was calculated as follows: the number of bacterial species divided by the sample size with the results expressed in percentages (Table 1). However, some studies used duplicates or replicate from the same fly homogenates. Thus, the number of positive bacterial species was recorded at 100% prevalence if the number recorded exceeds the sample size.

2.4 | Statistical analysis

Meta-analysis was carried out using Comprehensive Meta-Analysis Version 3.0. Between the studies, Cochran's heterogeneity (Q) within studies as well as percentage variation in prevalence was assessed using Higgin's I^2 (inverse variance) and Cochran's Q method. I^2 values of 25%, 50% and 75% were considered low, moderate and high heterogeneity, respectively (Higgins & Thompson, 2002). Subgroup analysis was also conducted by the location of study, study sites and diagnosis method used. The effect size and their corresponding confidence interval (95% CI) for each subgroup were calculated using MedCalc® statistical software and expressed on the forest plots. A funnel plot was generated to evaluate the potential for asymmetry or publication bias present.

3 | RESULTS

3.1 | Study selection

Following a systematic approach and bibliographic search, 1,978 references were retrieved from the initial search strategy (Figure 1). Citation searches were based on published journal articles. Overall, 1,133 studies were excluded following the initial screening based on titles or abstracts and duplicate journal articles were also uninvolved. The remaining 845 journal articles were further screened for

eligibility, and 767 studies were excluded for various reasons stipulated in Figure 1.

3.2 | Study characteristics of eligible studies

Characteristics of the eligible studies includes; the number of houseflies collected, bacterial species isolated, identification method, country and sampled sites as presented in Table S1. All the journal articles were published between the years 1980 and 2020. A handful of studies have been conducted globally that reports on bacterial pathogens isolated from houseflies. Studies included in this review were from four different continents namely; Africa, Americas, Asia and Europe (Figure 2). Asia (47.4%) was observed to have the highest number of studies followed by Americas (23.1%) and Africa (20.5%), and Europe (8.9%) being the least. The sample size from eligible studies ranged from 10 houseflies to 3,440 individual houseflies' specimens. Samples were collected from diverse sites including hospitals, water treatment facilities, slaughterhouses, garbage dumps, farms and residential areas. Thus, 38 (48.7%) studies were sampled from more than one site, while 39 (50%) of the studies sampled only from on one specific site and one (1.3%) study failed to report on their sampled site (Table S1).

The techniques employed by the eligible studies to isolate and identify bacterial species included culture-dependent (microbiological culture) and culture-independent (polymerase chain reaction [PCR]) respectively. However, other studies that made use of both techniques simultaneously were also included in the analysis. At least 56.4% (44/78) of the studies used culture method for the identification of pathogens and 30.7% (24/78) used the combination of both culture and PCR diagnostic technique for the identification and only 7.7% (6/78) used PCR method for the identification. However, one study failed to mention their diagnostic technique. Of 39/78 (50%) studies, reported on antibiotic susceptibility profile for different bacterial genera and only 13/78 (16.7%) studies reported on antibiotic-resistant genes. The bacterial species reported in this review are recognized pathogens including *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Amplification of some common resistance genes for colistin, sulphonamide, tetracycline, beta-lactam, penicillin, quinolones and integrons were observed in this study (Table S1).

3.3 | Meta-analysis

A total of 44 eligible studies were included in the quantitative synthesis (Table 1); this was owing to the study being definite with bacterial species. Substantial heterogeneity was observed between the eligible studies. The pooled estimates and heterogeneities of the eligible studies are presented in Tables 2–6 for the five selected pathogenic bacteria including *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. In all meta-analyses conducted, the overall effect estimates and their corresponding 95% confidence intervals (CI)

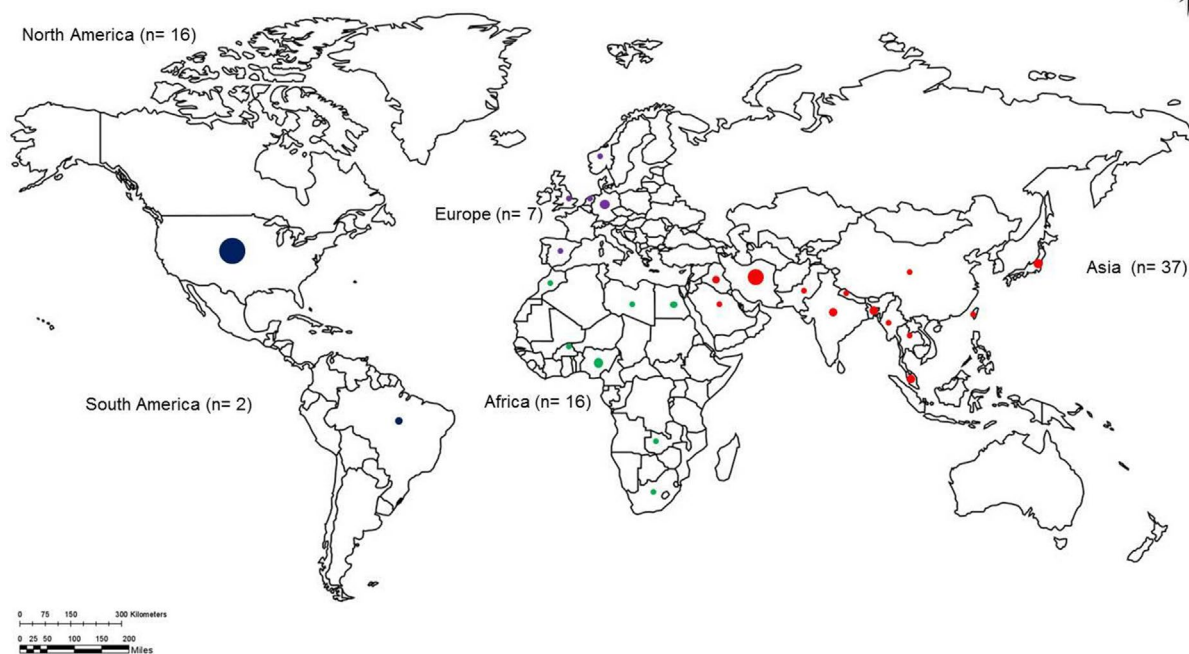


FIGURE 2 Number of countries, number of studies and sample size per continent. The size of the circle indicates the number of studies conducted in that country with different colours for different continents whereby green is for Africa, red for Asia, blue for North America and black is for South America

did not overlap point estimate (Figures 3–7). Similarly, the latter was observed in all the subgroups (Figures S1–S4).

3.3.1 | Pooling and heterogeneity estimates

Pooled proportions and their corresponding heterogeneities are presented in Tables 2–6 for *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* respectively. Substantial heterogeneity was observed for the selected pathogens as well as in the subgroup analysis. For *E. coli*, high heterogeneity was observed ($Q = 10,739.5523$; $I^2 = 99.60$; 95% CI: 99.57–99.63; $Q-p < 0.0001$; Table 2), followed by *S. aureus* ($Q = 2,439.1220$; $I^2 = 98.24$; 95% CI: 97.99–98.45; $Q-p < 0.0001$; Table 3), *P. aeruginosa* ($Q = 1,283.2227$; $I^2 = 96.65$; 95% CI: 96.07–97.15; $Q-p < 0.0001$; Table 4), *K. pneumoniae* ($Q = 1,576.6073$; $I^2 = 97.27$; 95% CI: 96.83–97.65; $Q-p < 0.0001$; Table 5) and *E. faecium* ($Q = 317.6145$; $I^2 = 86.46$; 95% CI: 82.70–89.41; $Q-p < 0.0001$; Table 6) being the least. The forest plot depicting the point estimate for individual studies reporting the occurrence of *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* are graphically presented (Figures 3–7).

3.3.2 | Subgroup analysis

Subgroup analyses were done for study locations (Africa, America, Asia and Europe), study sites (Dumping sites, farms, hospitals, market, no specific location, restaurant, slaughterhouse (Abattoir), university campus/residential houses and wastewater/broken sewage),

and diagnosis methods used (culture and molecular techniques [PCR]). Different subgroup analysis was undertaken for the five selected bacteria. The forest plot showing the point estimates results of the subgroup analysis are depicted in Figures S1–S4.

3.3.3 | Prevalence based on study region

High degree of heterogeneity with respect to *E. coli* was observed in four continents, whereas other pathogens including *P. aeruginosa* and *S. aureus* were observed in both Africa and Asia while *E. faecium* was observed only in America (Tables 2–6). With regards to *E. coli* identified in Africa, a high degree of heterogeneity was observed ($Q = 3,569.6776$; $I^2 = 99.72$; 95% CI: 99.68–99.76; $Q-p < 0.0001$) followed by America with ($Q = 2,599.8385$; $I^2 = 99.69$; 95% CI: 99.64–99.74; $Q-p < 0.0001$), while the least observed was in Asia ($Q = 1,487.0151$; $I^2 = 98.59$; 95% CI: 98.32–98.81; $Q-p < 0.0001$) (Table 2). The results were at variance for *S. aureus* and *P. aeruginosa* with high heterogeneity ($Q = 1,414.2219$; $I^2 = 98.52$; 95% CI: 98.23–98.76; $Q-p < 0.0001$) and ($Q = 772.7609$; $I^2 = 97.28$; 95% CI: 96.63–97.81; $Q-p < 0.0001$) in Asia.

3.3.4 | Prevalence based on sampling locations

High heterogeneity was found among studies investigating different pathogens including *E. coli*, *E. faecium*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa* from different sampling locations (Tables 2–6). The sampling locations include the farms, hospital, dumping site, market,

TABLE 2 Proportion of *Escherichia coli* isolated from houseflies sampled from regions, screening methods and sampling locations

	Number of studies	Pooled estimate		Measure of heterogeneity		Significance level Q-p
		Sample size	Positive	Q	I ² (95% CI)	
Bacterial species						
<i>Escherichia coli</i>	44	20,455	2,711	10,739.5523	99.60 (99.57–99.63)	<0.0001
Region						
Africa	11	13,581	875	3,569.6776	99.72 (99.68–99.76)	<0.0001
America	9	5,097	41	2,599.8385	99.69 (99.64–99.74)	<0.0001
Asia	22	196	1,003	1,487.0151	98.59 (98.32–98.81)	<0.0001
Europe	2	2	717	279.5474	99.64 (99.42–99.78)	<0.0001
Sampling locations						
Dumping sites	3	9,906	17	86.2819	97.68 (95.57–98.79)	<0.0001
Farms	18	1,705	797	2,075.0757	99.18 (99.04–99.30)	<0.0001
Hospitals	13	634	213	738.0189	98.37 (97.93–98.72)	<0.0001
Market	4	393	351	40.9600	92.68 (84.47–96.55)	<0.0001
No specific location	3	509	155	13.6833	85.38 (57.08–95.02)	=0.0011
Restaurant	6	493	245	6,303.3,062	99.75 (99.72–99.77)	<0.0001
Slaughterhouse (abattoir)	7	253	94	206.0165	97.09 (95.62–98.06)	<0.0001
University campus/ residential houses	7	426	186	134.0283	95.52 (92.86–97.19)	<0.0001
Wastewater/broken sewage	2	388	9	35.1989	97.16 (92.69–98.90)	<0.0001
Diagnostic technique						
Culture	25	319	1,537	2,698.8736	99.11 (98.98–99.23)	<0.0001
PCR	2	549	114	173.0078	99.42 (98.97–99.67)	<0.0001
Culture & PCR	17	11,587	985	6,303.3062	99.75 (99.72–99.77)	<0.0001

Abbreviations: CI, confidence interval; I², inverse variance; PCR, polymerase chain reaction; Q-p, Cochran's.

restaurant, slaughterhouse/abattoir, university campus/residential houses and wastewater/ broken sewage. High heterogeneity was observed (I² > 75%) among the studies, with studies investigating *E. coli* and *P. aeruginosa* from the farms having the highest heterogeneity (Q = 2,075.0757; I² = 99.18; 95% CI: 99.04–99.30; p < 0.0001) and (Q = 2,075.0757; I² = 99.18; 95% CI: 99.04–99.30; p < 0.0001), respectively. Also, high heterogeneity was observed from the studies investigating *E. faecium* from wastewater/broken sewage (Q = 13.7057; I² = 92.70; 95% CCI: 75.50–97.83; Q-p < 0.0001), respectively. However, studies investigating *S. aureus* in slaughterhouse/abattoir had moderate heterogeneity (Q = 13.5396; I² = 55.69; 95% CI: 0.00–80.97; Q-p < 0.0352) and studies investigating *K. pneumoniae* in university campus/residential houses had moderate heterogeneity (Q = 17.1543; I² = 65.02; 95% CI: 21.35–84.45; Q-p < 0.0087).

3.3.5 | Prevalence based on diagnostic methods

Two different diagnostic methods were used by the eligible studies to identify pathogens from houseflies, the combination of two diagnostic methods; culture and PCR had the highest heterogeneity

in studies investigating *E. coli* and *P. aeruginosa* (Q = 6,303.3062; I² = 99.75; 95% CI: 99.72 – 99.77; Q-p < 0.0001) and (Q = 490.0658; I² = 96.74; 95% CI: 95.76–97.48; Q-p < 0.0001). Lowest heterogeneity (Q = 25.1695; I² = 36.43; 95% CI: 0.00–64.46 Q-p = 0.0669) was observed with the studies investigating *E. faecium* using both culture and PCR techniques; Tables 2–6).

3.3.6 | Publication bias

According to the data observed from the funnel plot by virtual inspection, for all bacterial species, namely, *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, no publication bias was observed as the funnel was symmetrical (Figure 8). Thus, there was significant asymmetry for the analysis of all the selected bacterial pathogens.

4 | DISCUSSION

This study undertook a systematic review and meta-analysis to estimate the pooled prevalence of the occurrence of pathogenic bacterial

TABLE 3 Proportion of *Staphylococcus aureus* isolated from houseflies sampled from regions, screening methods and sampling locations

	Number of studies	Pooled estimate		Measure of heterogeneity		Significance level Q-p
		Sample size	Positives	Q	I ² (95% CI)	
Bacterial species						
<i>Staphylococcus aureus</i>	44	20,455	561	2,439.1220	98.24 (97.99–98.45)	<0.0001
Region						
Africa	11	13,581	56	230.0741	95.65 (93.77–96.97)	<0.0001
Asia	22	196	505	1,414.2219	98.52 (98.23–98.76)	<0.0001
Sampling locations						
Dumping sites	3	9,906	7	33.2093	93.98 (85.80–97.47)	<0.0001
Farms	18	1705	132	777.4544	97.81 (97.27–98.25)	<0.0001
Hospitals	13	634	31	79.6692	84.94 (75.80–90.62)	<0.0001
Market	4	393	4	17.0149	82.53 (54.61–93.15)	=0.0007
No specific location	3	509	12	38.1214	94.75 (88.02–97.70)	<0.0001
Restaurant	6	493	37	518.4722	96.91 (96.01–97.61)	<0.0001
Slaughterhouse (abattoir)	7	253	3	13.5396	55.69 (00.00–80.97)	=0.0352
University campus/ residential houses	7	426	44	42.3304	85.83 (72.83–92.61)	<0.0001
Wastewater/broken sewage	2	388	6	22.6646	95.59 (87.18–98.48)	<0.0001
Diagnostic technique						
Culture	25	319	447	1,603.7790	98.50 (98.23–98.73)	<0.0001
Culture & PCR	17	11,587	114	518.4722	96.91 (96.01–97.61)	<0.0001

Abbreviations: CI, confidence interval; I², inverse variance; PCR, polymerase chain reaction; Q-p, Cochran's.

TABLE 4 Proportion of *Pseudomonas aeruginosa* isolated from houseflies sampled from regions, screening methods and sampling locations

	Number of studies	Pooled estimate		Measure of heterogeneity		Significance level Q-p
		Sample size	Positives	Q	I ² (95% CI)	
Bacterial species						
<i>Pseudomonas aeruginosa</i>	44	20,455	299	1,283.2227	96.65 (96.07–97.15)	<0.0001
Region						
Africa	11	13,581	12	52.8707	81.09 (67.19–89.10)	<0.0001
Asia	22	196	287	772.7609	97.28 (96.63–97.81)	<0.0001
Sampling locations						
Dumping sites	3	9,906	5	23.9932	91.66 (78.72–96.74)	<0.0001
Farms	18	1,705	53	235.3866	92.78 (90.03–94.77)	<0.0001
Hospitals	13	634	107	282.6736	95.75 (94.12–96.94)	<0.0001
Slaughterhouse (abattoir)	7	253	7	51.2794	88.30 (78.30–93.69)	<0.0001
University campus/ residential houses	7	426	22	31.5330	80.97 (61.56–90.58)	<0.0001
Wastewater/broken sewage	2	388	4	14.9064	93.29 (78.01–97.95)	=0.0001
Diagnostic technique						
Culture	25	319	246	595.1690	95.97 (94.94–96.79)	<0.0001
Culture & PCR	17	11,587	53	490.0658	96.74 (95.76–97.48)	<0.0001

Abbreviations: CI, confidence interval; I², inverse variance; PCR, polymerase chain reaction; Q-p, Cochran's.

TABLE 5 Proportion of *Klebsiella pneumoniae* isolated from houseflies sampled from regions, screening methods and sampling locations

	Number of studies	Pooled estimate		Measure of heterogeneity		Significance level Q-p
		Sample size	Positives	Q	I ² (95% CI)	
Bacterial species						
<i>Klebsiella pneumoniae</i>	44	20,455	279	1,576.6073	97.27 (96.83–97.65)	<0.0001
Region						
Africa	11	13,581	17	79.1374	87.36 (79.31–92.28)	<0.0001
Asia	22	196	262	1,314.0651	98.40 (98.09–98.67)	<0.0001
Sampling locations						
Farms	18	1,705	51	262.1432	93.51 (91.14–95.26)	<0.0001
Hospitals	13	634	164	487.8350	97.54 (96.76–98.13)	<0.0001
Restaurant	6	493	2	628.8196	97.46 (96.76–98.00)	<0.0001
Slaughterhouse (abattoir)	7	253	37	91.3,336	93.43 (88.91–96.11)	<0.0001
University campus/ residential houses	7	426	4	17.1543	65.02 (21.35–84.45)	=0.0087
Diagnostic technique						
Culture	25	319	211	889.4596	97.30 (97.70–97.79)	<0.0001
Culture & PCR	17	11,587	68	628.8196	97.46 (96.76–98.00)	<0.0001

Abbreviations: CI, confidence interval; I², inverse variance; PCR, polymerase chain reaction; Q-p, Cochran's.

TABLE 6 Proportion of *Enterococcus faecium* isolated from houseflies sampled from regions, screening methods and sampling locations

	Number of studies	Pooled estimate		Measure of heterogeneity		Significance level Q-p
		Sample size	Positive	Q	I ² (95% CI)	
Bacterial species						
<i>Enterococcus faecium</i>	44	20,455	77	317.6145	86.46 (82.70–89.41)	<0.0001
Region						
Americas	9	1,581	77	178.3214	95.51 (93.27–97.01)	<0.0001
Sampling locations						
Dumping sites	3	9,906	4	20.4559	90.22 (74.03–96.32)	<0.0001
Farms	18	1,705	3	15.6957	00.00 (00.00–45.86)	=0.5455
Restaurant	17	11,587	14	25.1695	36.43 (00.00–64.46)	=0.0669
Wastewater/broken sewage	2	388	60	13.7057	92.70 (75.50–97.83)	=0.0002
Diagnostic technique						
Culture	25	7,970	60	228.5404	89.85 (86.19–92.54)	<0.0001
PCR	2	549	14	30.1038	96.68 (91.08–98.76)	<0.0001
Culture & PCR	17	11,587	3	25.1695	36.43 (00.00–64.46)	=0.0669

Abbreviations: CI, confidence interval; I², inverse variance; PCR, polymerase chain reaction; Q-p, Cochran's.

species of medical and veterinary importance carried by houseflies globally. The number of pathogens reported by the studies for qualitative synthesis varied greatly with most reporting on pathogenic and virulent bacterial strains. Thus, the pathogenic Gram-negative bacteria were by far the most frequent with most studies as compared to Gram-positive bacteria. Different bacterial species found on houseflies depend on the location where the insects are captured (Cervelin et al., 2018). This study has also proved that houseflies are in abundance in different

settings and geographical locations. These findings are in accordance with the review by Issa (2019) where they indicated that houseflies live closely with humans and domestic animals and often found in areas of human activities such as restaurants, hospitals, food centres, food markets, fish markets and slaughterhouses. With the available information to date, there is evidence that houseflies are moderate to high in various human and animal settings harbouring clinically and veterinary relevant bacterial species in abundance (Gupta et al., 2012).

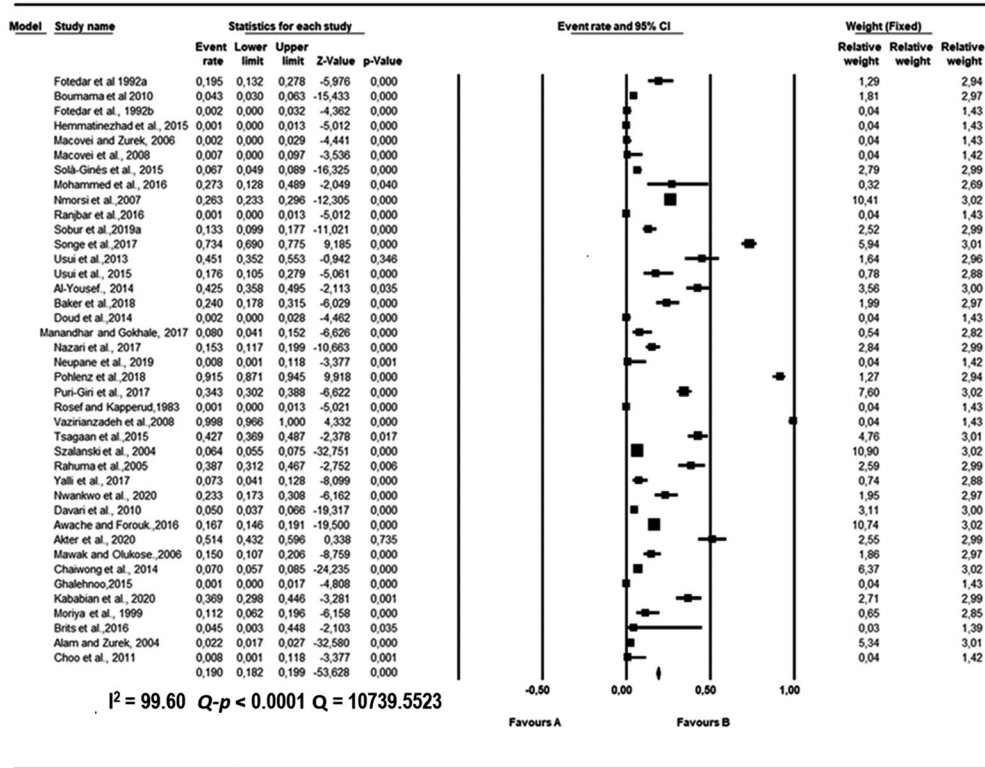


FIGURE 3 Forest plot showing the pooled estimates of *Escherichia coli* proportion isolated from houseflies. The squares demonstrate the individual point estimate. The diamond at the base indicates the pooled estimates from the overall studies

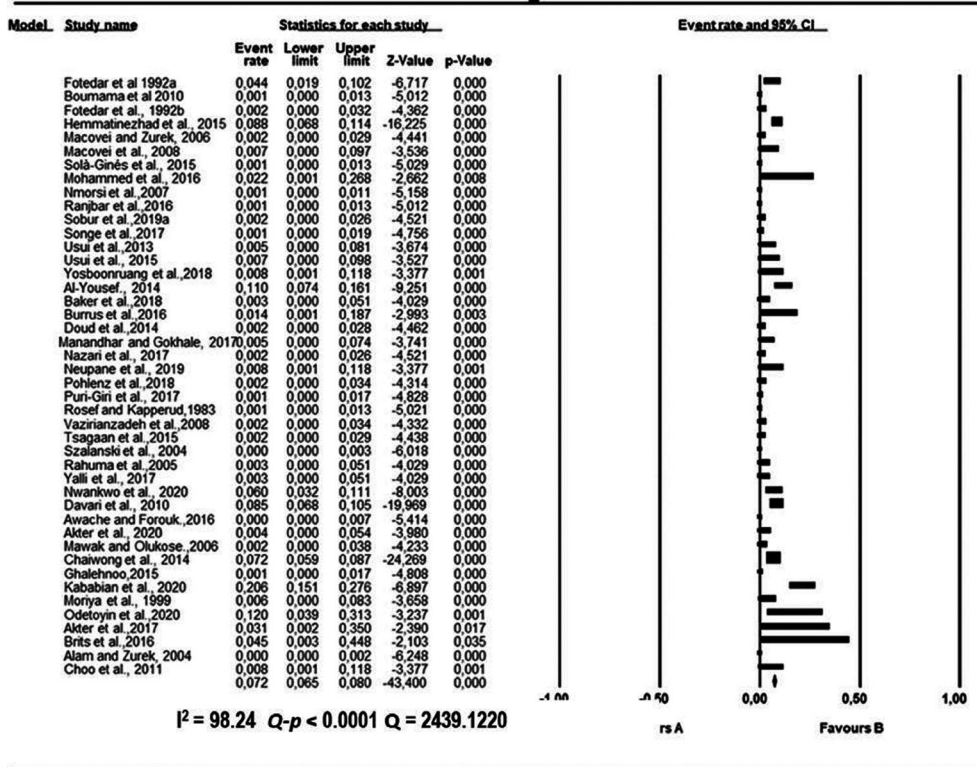


FIGURE 4 Forest plot showing the pooled estimates of *Staphylococcus aureus* proportion isolated from houseflies. The squares demonstrate the individual point estimate. The diamond at the base indicates the pooled estimates from the overall studies

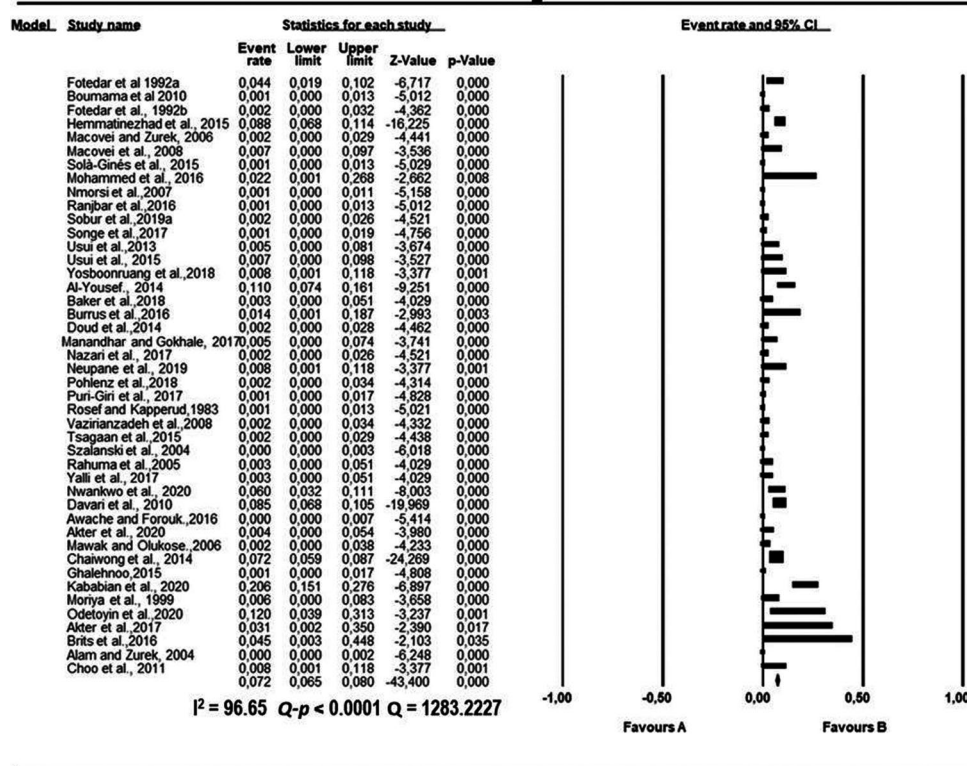


FIGURE 5 Forest plot showing the pooled estimates of *Pseudomonas aeruginosa* proportion isolated from houseflies. The squares demonstrate the individual point estimate. The diamond at the base indicates the pooled estimates from the overall studies

M. domestica is most frequently associated with zoonoses worldwide (Wales et al., 2010). Through history, housefly colonization by bacteria has been reported previously by other studies including the study by Moorhead and Weisser (1946) where they recorded and proved the survival of *Staphylococcus* strains in the gut and excreta of houseflies. Additionally, other studies also documented that *M. domestica* is becoming heavily colonized by various bacterial genera including *Staphylococci*, *Streptococci* and *Salmonellae* (Mawak & Okulose, 2006). Thus, houseflies have been implicated as vectors or transporters of various human pathogens, including *Vibrio cholerae*, Enterobacteriaceae pathogens, *S. aureus* and *Pseudomonas* spp. (Ahmed et al., 2013; Davari et al., 2010).

The most frequent reported bacterial species by various studies were the following: (a) *E. coli*, the bacterium that causes nosocomial infections and bloody diarrhoea and haemorrhagic colitis in humans (Blaak et al., 2014; Iwasa et al., 1999; Poudel et al., 2019; Wales et al., 2010); (b) *E. faecium*, the most important nosocomial pathogens of humans, causing urinary tract infections, bloodstream infections, endocarditis and wound infections (Hammerum, 2012; Macovei et al., 2008; Selleck et al., 2019); (c) *K. pneumoniae*, which cause infections of the respiratory tract and urinary tract, as well as post-operative wound infections (Blaak et al., 2014; Ranjbar et al., 2016); (d) *P. aeruginosa*, cause disease in humans and other animals (Banjo et al., 2005); and (e) *S. aureus* which is capable of causing human illness and food poisoning (Banjo et al., 2005).

Antimicrobial resistance results in a serious public health problem, for it weakens the treatment efficacy against bacterial

infections and this poses greater health risks (Russell et al., 2018; Wei et al., 2013). Some studies have reported some bacterial isolates which are resistant to significantly more than one of the commonly used antibiotics tested (Akter et al., 2020; Hemmatinezhad et al., 2015; Nazari et al., 2017; Nwankwo et al., 2020; Odofin et al., 2020; Rahuma et al., 2005). According to Russell et al. (2018), dipterans that associate with livestock, livestock waste products and cadavers have the potential to acquire livestock-associated antibiotic resistant bacteria and transmit them to humans. In the year 2017, the WHO made a list of antibiotic-resistant priority pathogens which present a great threat to humans (WHO, 2017). Gram-negative bacterial pathogens including *E. coli*, *K. pneumoniae*, *P. aeruginosa* and Gram-positive *E. faecium* and *S. aureus* were the most listed under the critical and high categories with regard to urgent need of new antibiotics (WHO, 2017). Owing to the distinctive structure amongst the Gram-negative and Gram-Positive bacteria, the Gram-negative bacteria appear to be more resistant to antibiotics and cause significant morbidity and mortality worldwide (Breijyeh et al., 2020).

Release of antibiotic resistance bacteria expressing antibiotic resistance genes into the environment creates an opportunity for other insects to acquire livestock-associated antibiotic-resistant bacteria (Russell et al., 2018). Some studies in this review have reported the presence of resistance genes including mobile colistin, tetracycline and extended-spectrum β -lactamase gene, from bacterial isolates identified from houseflies. This raises a very great concern since colistin is considered a last-resort antimicrobial for treating patients infected with multidrug-resistant Gram-negative

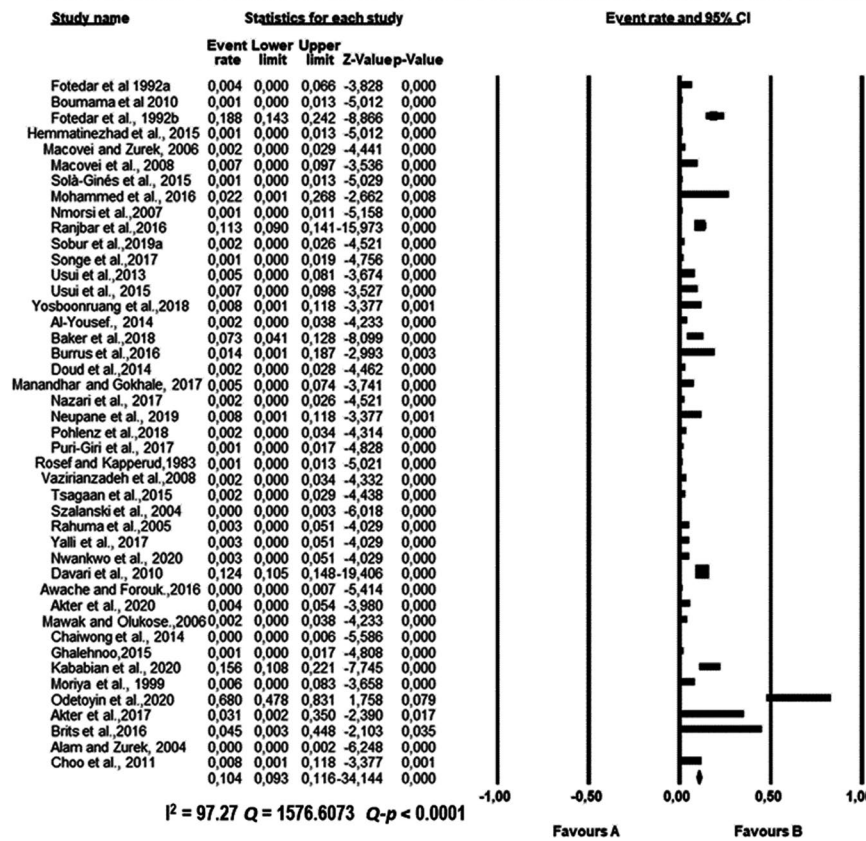
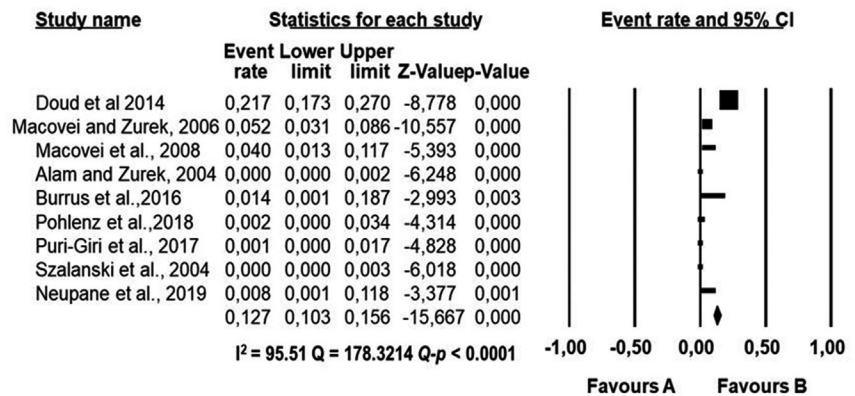


FIGURE 6 Forest plot showing the pooled estimates of *Klebsiella pneumoniae* proportion isolated from houseflies. The squares demonstrate the individual point estimate. The diamond at the base indicates the pooled estimates from the overall studies

FIGURE 7 Forest plot showing the pooled estimates of *Enterococcus faecium* proportion isolated from houseflies. The squares demonstrate the individual point estimate. The diamond at the base indicates the pooled estimates from the overall studies



bacteria (Zhang et al., 2018). The spreading of the plasmid encoded extended-spectrum β -lactamase gene which can confer resistance to third generation cephalosporins is the major problem because third generation cephalosporins are clinically important antimicrobials in human and veterinary medicine (Usui et al., 2013). A review by Onwugamba et al. (2018) on the role of flies in the spread of antimicrobial resistance also revealed that ‘filth flies’ are colonized by clinically important antimicrobial resistant bacteria, including extended-spectrum beta-lactamase, carbapenemase-producing, and colistin-resistant bacteria. The presence of these resistant genes from the bacterial isolates simply indicates that houseflies have the

capacity to significantly disseminate bacteria carrying antibiotic resistant genes from various environmental settings to humans (Zhang et al., 2018).

In this study, we observed high variance in the number of studies conducted on bacterial pathogens harboured by houseflies per continent. Thus, Asia had the highest number of eligible studies followed by America, Africa and Europe, respectively. The large number of studies in Asia can be due to the fact that, houseflies originate from Central Asia (Sanchez-Arroyo & Capinera, 2015). However, they are now common in all climates ranging from tropical regions to temperate climates (Khamesipour et al., 2018; Sanchez-Arroyo &

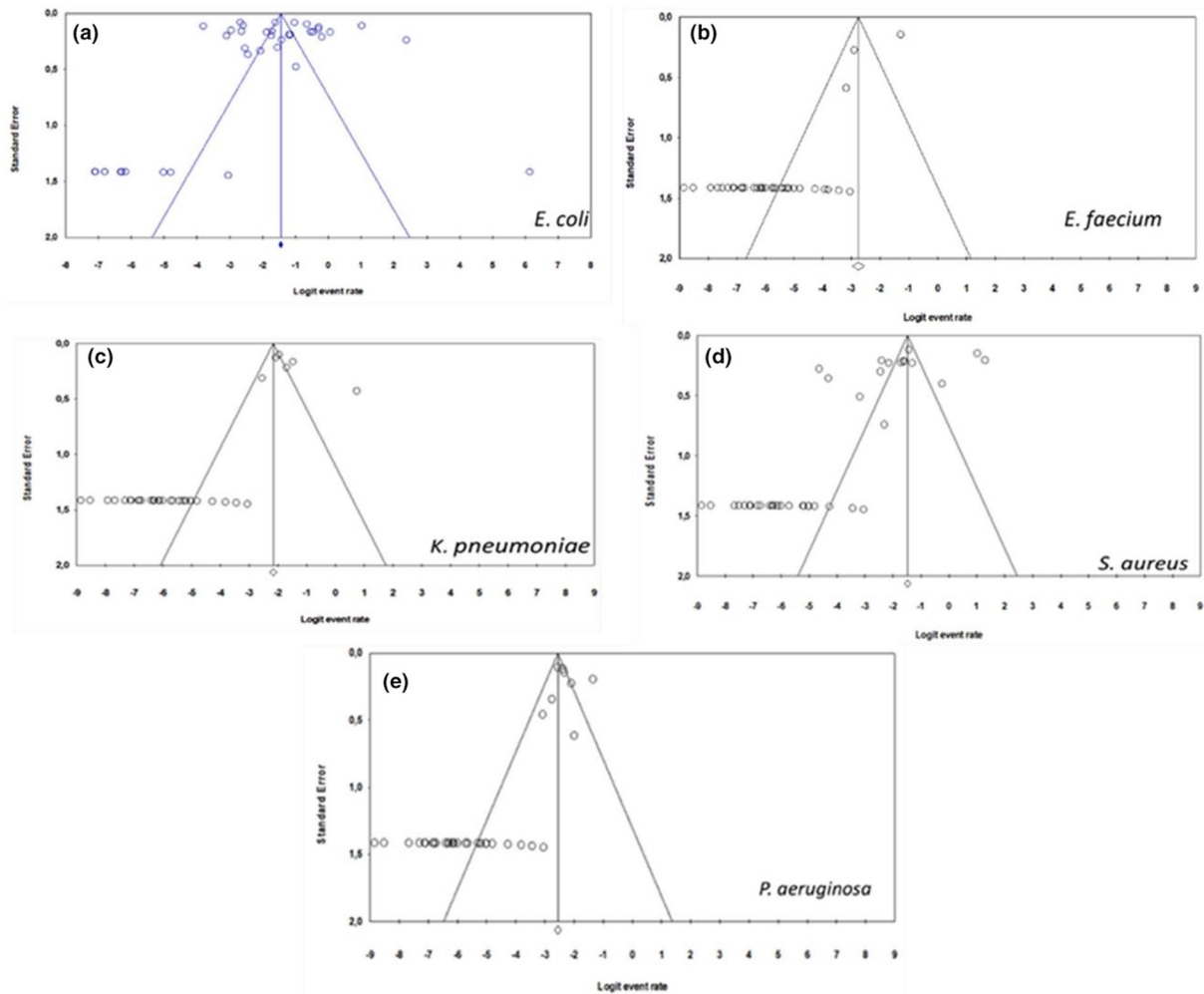


FIGURE 8 Funnel plots for studies investigating: (a) *E. coli*, (b) *E. faecium*, (c) *K. pneumoniae*, (d) *S. aureus* and (e) *P. aeruginosa*

Capinera, 2015). Hence there's a large number of studies observed from other continents including America (Alves et al., 2018; Burrus et al., 2017; Macovei et al., 2008; Macovei & Zurek, 2006; Olsen & Hammack, 2000; Poudel et al., 2019, Africa (Brits et al., 2016; Ibrahim et al., 2018; Nwankwo et al., 2020; Songe et al., 2016) and Europe (Blaak et al., 2014; Davies et al., 2014; Rosef & Kapperud, 1983; Solà-Ginés et al., 2015).

Culturing and plating conventional microbiological methods are still regarded as a gold standard, as they enable the identification of various bacterial species effectively (Kotsilkov et al., 2015). However, the combination of both microbiological culturing and molecular methods is increasingly adopted (Langley et al., 2015). Due to the use of different pathogen detection methods (PCR and culture including selective media and/or broth enrichment), the bacterial carrier rates from flies must be cautiously compared (Onwugamba et al., 2018). Most of the studies in this review used selective culture media to identify predominantly pathogenic bacteria or specific species. However, medium preferences and growth condition optima of individual bacterial species may influence the observation of species diversity and the abundance of individual bacteria isolated (Brits et al., 2016). The combination of both culture and molecular

techniques has shown to improve the identification and isolation rate of bacterial species. However, the advanced method including PCR is costly (Khamesipour et al., 2018). A review by Adzeity et al. (2013) showed that studies using molecular methods (PCR and/or sequences) yield more pathogens compared to studies using standard cultural methods. However, Gupta et al. (2012) showed that there are benefits and limitations concerning the use of either approach for diagnosis.

Notably, all studies included in this systematic review and meta-analysis were conducted differently, as some studies investigated large number of samples while others investigated different settings and utilizing various diagnostic techniques. These parameters resulted in the high heterogeneity observed in this study. According to Opong et al. (2020), heterogeneity could partially be attributed to large variation in sampling and diagnostic techniques applied for the detection of the bacterial pathogens. This is because different techniques differ in their detection sensitivity, specificity, as well as positive and negative predictive values. There was no statistical significance at the study level, but we observed statistical significance at the meta-analysis. Substantial heterogeneity was observed in studies included in our analyses which can largely be attributed to

variation in sample size, sampling method and analysis of data. Some studies presented small sample size resulting in effect estimates with wide confidence intervals.

This study has considerable number of strengths including (a) this study summarizes currently existing knowledge and helps in identifying areas that lack adequate evidence, thereby producing new research questions. (b) We have checked the overall consistency of the searching process, study choice and inclusion/exclusion criteria. All the duplicated records were removed. The authors independently examined the titles, abstracts and full-text article. This was done in order to negate any possibility of errors. (c) The application of a consistent reviewing approach and extracting relevant data from the available published studies which allowed to reliably determine the pathogenic bacteria isolated from houseflies by various studies around the world and how frequent these studies report on the antibiotic susceptibility profiles and resistant genes in common bacterial pathogens in different countries (d) exclusion of duplicate studies and studies that are not specific with genus and species of the fly, studies using laboratory bred houseflies while including only studies with genus and species confirmed (e) and considering studies with samples collected from different human and animal settings.

Several limitations have been identified which included the following: (a) only journal papers published in English language were considered. This was due to lacking resources for translation of papers that might have been published in other languages. (b) Few studies have failed to describe the sample size despite the importance of considering the sample size in all investigational studies. Sample size calculation is ethically important and form part of the early stages of leading an epidemiological, clinical or laboratory studies (Faber & Fonesca, 2014). (c) Furthermore, some countries had more research reports compared to others.

In conclusion, this systematic review and meta-analysis has provided global overview on the carriage of significant bacterial pathogens including antibiotic resistant bacteria identified in houseflies from different regions in the world. Houseflies are in abundance and found almost in every human and animal habitation and this pose a serious threat to public health. Most of the studies in this review predominantly identified pathogenic bacteria including *E. coli*, *E. faecium*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus*. Few studies presented data on antibiotic susceptibility profile and resistant genes from housefly, and this is of great concern, as flies have the potential to rapidly and widely disseminate antimicrobial resistant genes. Studies in this review were found to employ different diagnostic methods including culture and PCR or the combination of both techniques. Further studies with special focus on antibiotic susceptibility profile and resistant genes from bacteria identified from houseflies will provide more insight global threat to human and animal health (Zhang et al., 2018). Future systematic reviews may also include the following: (a) data from different life cycles of housefly including eggs, larvae and pupa (b) data from culture-independent studies that allow the identification of the cultivable and non-culturable fractions of bacteria present in houseflies. The findings of this study provide more insight on the carriage of significant bacterial pathogens

of distinct taxonomic identities by *M. domestica* from different geographical locations across the world. Continuous investigation is required to completely comprehend the significance of medically and veterinary important pathogenic bacteria harboured by houseflies. Also, further investigation is needed to quantify the contamination level in order to analyse the risk of bacterial contamination and dissemination by houseflies.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in relation to this review paper.

AUTHOR CONTRIBUTION

Maropeng Charles Monyama: Conceptualization; Data curation; Formal analysis; Investigation; Writing-original draft; Writing-review & editing. **Emmanuel ThankGod Onyiche:** Methodology; Validation; Writing-review & editing. **Moeti Oriel Taioe:** Validation; Writing-review & editing. **Jane Nkhebenyane:** Validation; Writing-review & editing. **Oriel Matlhahane Molifi Thekiso:** Conceptualization; Supervision; Validation; Writing-review & editing.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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