PREVALENCE AND ANTIMICROBIAL RESISTANCE OF PATHOGENIC BACTERIA IN ANIMAL FOOD PRODUCTS IN CENTRAL PENINSULAR MALAYSIA

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ABSTRACT. Antimicrobial resistance (AMR) represents a major global health concern, and using antimicrobials in animals raised for food production could potentially intensify this challenge. This research investigates the prevalence of AMR in *Salmonella* spp. and *Escherichia coli* (*E. coli*) isolated from food of animal origin in central Peninsular Malaysia. A total of 3,012 samples were collected from Melaka, Negeri Sembilan, Putrajaya, and Selangor in 2022, isolating 76 *Salmonella* spp. (2.5%) and 230 *E. coli* (7.6%). These samples consisted of beef/buffalo meat, beef products, chicken meat, chicken products, edible bird's nests, and fermented milk. Antimicrobial susceptibility was assessed using disk diffusion and broth dilution techniques. Both pathogens demonstrated resistance to various antimicrobials, including ampicillin, tetracycline, streptomycin, sulfamethoxazole/trimethoprim, chloramphenicol, gentamicin, ciprofloxacin, cefotaxime, ceftiofur, and colistin sulphate. Resistance rates fluctuated, with ampicillin exhibiting the highest resistance (66.7% for *Salmonella* spp. and 81.7% for *E. coli*), while colistin sulphate had the lowest resistance (1.3% and 3%, respectively). The greatest sensitivity for both pathogens was observed with ceftiofur (88.2% for *Salmonella spp.* and 76.5% for *E. coli*). Multi-drug resistance was present in 64% of *Salmonella* spp. and 78.7% of *E. coli* isolates. The highest prevalence of these pathogens was detected in beef/buffalo meat (32% for *Salmonella spp.* and 48.7% for *E. coli*) and chicken meat (9.3% for *Salmonella spp.* and 44.3% for *E. coli*). These outcomes underscore the significant incidence of AMR in animal-derived foods in the studied region, offering critical data for assessing foodborne AMR risks and preventing related illnesses. The findings highlight the need to monitor and regulate antimicrobial use in food-producing animals to curb the spread of AMR bacteria through the food supply chain.

***Keywords*:** antimicrobial resistance, *Salmonella spp*, *E. coli*, animal food products, Peninsular Malaysia

INTRODUCTION

Antimicrobial resistance (AMR) represents a critical threat to global public health, which compromises the effective treatment of infections and destabilises healthcare systems. AMR refers to the capacity of microorganisms to resist the action of antibiotics and other antimicrobial agents, thereby complicating the treatment of diseases in humans, animals, and plants. This phenomenon presents major challenges to both public health and food security (Loftus *et al*., 2020).

In light of the grave consequences associated with AMR, international organisations have initiated coordinated responses. In 2015, the World Health Assembly endorsed the Global Action Plan on AMR, a collaborative effort involving the Food and Agriculture Organization (FAO), World Health Organization (WHO), and World Organisation for Animal Health (OIE) (WHO, 2015). At the heart of this initiative was the 'One Health' approach, which highlighted the interconnectedness of human, animal, and environmental health in addressing AMR (FAO, 2021). Over the past two decades, antibiotic consumption in humans has escalated, with particular concern on increasing usage of last-resort antibiotics. The correlation between human antibiotic consumption and rising resistance rates in pathogenic bacteria has been observed across diverse settings, elevating AMR as a top priority for governments, international agencies, and global One Health initiatives (Walsh *et al*., 2023).

The impact of AMR is particularly acute in low- and middle-income countries (LMICs), where the absence of robust surveillance systems and regulatory frameworks governing antimicrobial use exacerbates the problem. Key areas for intervention include the development of National Action Plans, the enhancement of laboratory surveillance capabilities, and the implementation of educational programs targeting both healthcare professionals and the broader public (Loftus *et al*., 2020; Mendelsohn *et al*., 2023).

In alignment with global AMR initiatives, Malaysia established the National Antimicrobial Resistance Committee (NARC) in 2017. This multi-sectoral body coordinates national efforts through four technical working groups dedicated to raising awareness, conducting surveillance, preventing infections, and promoting the judicious use of antimicrobials (MyAP-AMR, 2017-2021).

Salmonella spp. and *E. coli* are among the most prevalent foodborne pathogens, and their capacity to acquire and transmit antimicrobial resistance within the food chain is of particular concern. The widespread use of antimicrobials in food-producing animals heightens the risk, especially considering that the volume of antibiotics used in livestock production far exceeds those used in humans and is projected to rise globally, particularly in developing economies (Milho *et al*., 2021; Mendelsohn *et al*., 2023).

Despite coordinated efforts at both international and national levels, there is still lack of comprehensive data on the prevalence of AMR in foodborne pathogens in Malaysia. To address this knowledge gap and enhance AMR surveillance in the food sector, this study seeks to determine the prevalence of antimicrobial-resistant *Salmonella* spp. and *E. coli* isolated from animal food products in central Peninsular Malaysia. The findings of this research will provide crucial data to assist targeted interventions and policy strategies in the ongoing efforts to combat AMR.

MATERIALS AND METHOD

Sample Collection and Processing

In this study, we evaluated data from the National Food Safety Monitoring Program (NFSMP), which include analyses of 3,012 food samples of animal origin collected between January and November 2022. Meat inspectors gathered the samples from Veterinary Health Mark (VHM) plants, Good Veterinary Hygiene Practice (GVHP) premises, and slaughterhouses, all overseen by the Department of Veterinary Services (DVS) in central Peninsular Malaysia, covering Melaka, Negeri Sembilan, Putrajaya, and Selangor.

Bacterial Isolation and Identification

E. coli enumeration was performed according to the Bacteriological Analytical Manual of the Food and Drug Administration (FDA BAM, Chapter 4: 2020). *Salmonella* spp. isolation was conducted following the Australian Standard (AS 5013.10; 2022).

A weight of 25 g sample was pre-enriched in buffered peptone water (BPW) at a 1:10 dilution and homogenized for approximately 60 s using a stomacher (BagMixer 400, Interscience, France). The homogenate was then incubated and cultured on chromogenic selective agar. For *E. coli* and coliform count, Molten Brilliance Agar

(Oxoid, UK) was used. For *Salmonella* spp., XLT4 (Oxoid, UK) and Rambach Agar (Merck, Germany) were used. Colonies were examined after the incubation period, and results were expressed as CFU/g in 25 g of sample.

Antimicrobial Susceptibility Testing

Two methods were employed for antimicrobial susceptibility testing:

a) Disk Diffusion (Kirby-Bauer) Test: A suspension of each isolate was prepared to a specific McFarland standard and spread evenly onto Mueller-Hinton or Iso-Sensitest agar (Oxoid, UK). Antibiotic-impregnated disks were placed on the agar surface. After incubation, zones of inhibition were measured and interpreted as susceptible (S), intermediately susceptible (I), or resistant (R) based on reference tables.
b) Broth Dilution Method: Isolates were inoculated in tubes or microtiter plates with antibiotic-containing broth media at varied doses (typically doubling dilutions). After incubation, turbidity was measured visually or using an automatic reader to determine the breakpoint concentration.

Calculation of Multiple Antimicrobial Resistance (MAR)

The Multiple Antimicrobial Resistance (MAR) Index is a quantitative method used to assess the resistance of bacterial isolates to multiple antimicrobial agents. It helps in identifying the risk level associated with the source of bacteria, where a higher MAR index indicates a higher exposure to antibiotics. Antimicrobial contamination was regarded as low risk when the MAR index was ≤ 0.2, and as high risk when it was ≥ 0.2 (Akande *et al*., 2019). The Multiple Antimicrobial Resistance (MAR) Index was calculated as reported by Christopher *et al*., 2013.

RESULT AND DISCUSSION

Table 1 summarises the prevalence and distribution of *Salmonella* spp. and *E. coli* isolated from animal food products in central Peninsular Malaysia. The prevalence rates of *Salmonella* spp. (2.5%) and *E. coli* (7.6%) in these foods indicate a notable presence of these pathogens in the food chain, although these figures are lower than those reported in some previous regional studies. For instance, Shafini *et al*. (2017) documented a prevalence of 40.4% for *Salmonella* spp. in raw chicken meat sold in retail outlets across Malaysia, suggesting potential improvements in hygiene practices or differences in sampling methodologies.

This study identified regional variations, with Selangor exhibiting the highest prevalence of *E. coli* at 9.2%, while Melaka had the highest prevalence of *Salmonella* spp. at 3.1%. These findings are consistent with those of Tung *et al*. (2016) and highlight how local environmental and management factors can significantly influence the distribution of these pathogens.

Additionally, these results are comparable to previous research conducted in Malaysia, where a study in the East Coast region reported a *Salmonella* spp. prevalence of 6.5% and an *E. coli* prevalence of 51.8% in broiler chickens, exceeding the *E. coli* prevalence found in current study. On a global scale, the prevalence of *Salmonella* spp. in poultry varies widely, ranging from as low as 2% in some European countries to as high as 48% in Bangladesh (Ibrahim *et al*., 2021).

Table 1. Prevalence and distribution of *Salmonella* spp. and *E. coli* isolated from animal food products from Central Peninsular Malaysia

Location	No. of samples tested	Prevalence of *Salmonella* spp.	Prevalence of *E. coli*
Melaka	193	6 (3.1%)	9 (4.7%)
Negeri Sembilan	474	12 (2.5%)	40 (8.4%)
Putrajaya	459	5 (1.1%)	6 (1.3%)
Selangor	1,886	53 (2.8%)	175 (9.2%)
Total	3,012	76 (2.5%)	230 (7.6%)

Table 2 presents the prevalence of *Salmonella* spp. and *E. coli* across different sample types in central Peninsular Malaysia. Notably, the highest prevalence of both pathogens was found in beef/buffalo meat, with rates of 32% for *Salmonella* spp. and 48.7% for *E. coli*. These figures are particularly alarming and exceed those reported in other countries, such as the 25% prevalence of *Salmonella* spp. in buffalo meat identified by Abd-Elghany *et al*. (2022) in Egypt. This significant discrepancy underscores the urgent need for implementing more stringent control measures in beef processing and handling to safeguard public health and mitigate the spread of antimicrobial-resistant bacteria.

Table 2. Prevalence of *Salmonella* spp and *E. coli* in different sample type in Central Peninsular Malaysia

Sample type	No. isolates of *Salmonella* spp.	Percent (%) of *Salmonella* spp. isolates	No. isolates of *E. coli*	Percent (%) of *E. coli* isolates
Beef / buffalo meat	24	32	112	48.7
Beef products	0	0	1	0.4
Chicken meat	36	48	102	44.3
Chicken products	7	9.3	3	1.3
EBN	4	5.3	3	1.3
Fermented milk	4	5.3	9	3.9
Total	75	100	230	100

Table 3 illustrates the antimicrobial susceptibility patterns of *Salmonella* spp. and *E. coli* isolates against various antimicrobial agents. Both pathogens displayed significant resistance to ampicillin, tetracycline, and streptomycin, which is consistent with global trends reported by the WHO (2021). However, the resistance rates observed in that study were notably higher than those reported in some developing countries (Jajere, 2019). Specifically, the isolates exhibited considerable resistance to commonly used antimicrobials, with resistance rates of 66.7%

for *Salmonella* spp. and 81.7% for *E. coli* against ampicillin, 61.3% and 75.7% against tetracycline, and 48% and 64.8% against streptomycin, respectively. These findings align with previous research conducted in the East Coast region of Peninsular Malaysia (Ibrahim *et al*., 2021), which also reported high resistance levels in *Salmonella* spp. and *E. coli* isolates from broilers. Similarly, elevated resistance levels have been documented in other countries, including Bangladesh (Rafiq *et al*., 2022).

Furthermore, the data indicate that *E. coli* exhibited higher resistance rates than *Salmonella* spp. for most antibiotics tested. For example, 46.1% of *E. coli* isolates were resistant to ciprofloxacin, while only 12% of *Salmonella* spp. were resistant. Additionally, resistance to chloramphenicol was more common in *E. coli* (61.3%) compared to *Salmonella* spp. (32%). On a positive note, the relatively lower resistance rates observed for ceftiofur and colistin sulphate in current study are encouraging, considering these antibiotics are classified as last-resort treatments.

Table 3. Susceptibility of *Salmonella* spp. and *E. coli* isolated from animal food products towards ten antimicrobials

Antimicrobial agents	Susceptible (S)		Intermediate (I)		Resistant (R)	
	Salmonella spp.	*E. coli*	*Salmonella* spp.	*E. coli*	*Salmonella* spp.	*E. coli*
AMP_10	25 (33.3%)	31 (13.5%)	0	11 (4.8%)	50 (66.7%)	188 (81.7%)
CTX_30	65 (86.7%)	155 (67.4%)	2 (2.7%)	23 (10%)	8 (10.7%)	52 (22.6%)
EFT_30	66 (88%)	176 (76.5%)	2 (2.7%)	14 (6.1%)	7 (9.3%)	40 (17.4%)
C_30	42 (56%)	83 (36.1%)	9 (12%)	6 (2.6%)	24 (32%)	141 (61.3%)
CIP_5	39 (52%)	100 (43.5%)	27 (36%)	24 (10.4%)	9 (12%)	106 (46.1%)
CT_10	35 (46.7%)	44 (19.1%)	0.0	0.0	1 (1.3%)	7 (3%)
CN_10	65 (86.7%)	151 (65.7%)	0.0	9 (3.9%)	10 (13.3%)	70 (30.4%)
S_10	26 (34.7%)	52 (22.6%)	13 (17.3%)	29 (12.6%)	36 (48%)	149 (64.8%)
SXT_25	46 (61.3%)	99 (43%)	1 (1.3%)	1 (0.4%)	28 (37.3%)	130 (56.5%)
TE_30	29 (38.7%)	53 (23%)	0.0	3 (1.3%)	46 (61.3%)	174 (75.7%)

Table 4 highlights the alarming trends in the analyses of multiple antimicrobial resistance (MAR) patterns and the multidrug resistance index (MDR) in *Salmonella* spp. and *E. coli* isolates obtained from animal food products in central Peninsular Malaysia. A notably high prevalence of multidrug resistance was observed, with 86.8% of *Salmonella* spp. and 90% of *E. coli* isolates demonstrating resistance to more than one antimicrobial agent. The most common resistance profile for *Salmonella* spp. involved resistance to a single antimicrobial (22.7%), while *E. coli* displayed more intricate resistance patterns, with 23% resistant to six different antimicrobials.

Interestingly, the MAR index, which evaluates the ratio of antibiotics to which an isolate is resistant compared to the total number of antibiotics tested, indicated that 64% of *Salmonella* spp. isolates and 78.7% of *E.*

coli isolates had values ≥ 0.2. This suggests that these bacteria were likely originated from high-risk environments where antibiotic use is frequent.

These findings are consistent with global trends reported in recent years, such as those noted by Jajere (2019), who found similar resistance patterns in food animals and their products across various regions. The resistance to critically important antimicrobials like ciprofloxacin and cefotaxime is particularly alarming, reflecting concerns raised by the WHO (2021) regarding the increasing resistance to fluoroquinolones and third-generation cephalosporins. The elevated levels of multidrug resistance and high MAR indices observed in this study highlight significant public health concerns about the transmission of antimicrobial resistance through the food chain, potentially resulting in difficult-to-treat infections in humans, as emphasised by reports from FAO/WHO/OIE (2018).

Table 4. Antimicrobial resistance patterns and multiple resistance index (MAR) in *Salmonella* spp. and *E. coli* isolates from animal food products

No. of antimicrobial	*Salmonella* isolates (n=75)		*E. coli* isolates (n=230)		MAR Index
	No. of isolates	MDR profile	No. of isolates	MDR profile	
1	17 (22.7%)	TE, CIP, S, AMP	26 (11.3%)	AMP, CIP, TE, S	0.1
2	7 (9.3%)	CIP-S, AMP-TE, AMP-S	10 (4.3%)	AMP-TE, AMP-CN, AMP-TE, AMP-S, CIP-TE C-CTX	0.2
3	11 (14.7%)	AMP-CIP-TE, CN-S-TE AMP-CN-S, AMP-CIP-SXT C-S-TE, CIP-S-TE, AMP-SXT-TE, AMP-S-TE	14 (6.1%)	AMP-S-TE, CIP-S-SXT, AMP-C-CIP, AMP-S-TE AMP-C-TE, AMP-CTX-TE, C-CN-TE, AMP-SXT-TE AMP-C-SXT, AMP-C-TE, AMP-CTX-EFT	0.3
4	15 (20%)	C-S-SXT-TE, AMP-C-S-TE AMP-CT-S-TE, AMP-C-S-SXT, AMP-S-SXT-TE, AMP-C-SXT-TE, AMP-CTX-EFT-TE	22 (9.6%)	AMP-S-SXT-TE, AMP-CIP-S-SXT, AMP-C-S-TE AMP-CIP-S-TE, AMP-C-SXT-TE, AMP-CTX-CIP-S AMP-CIP-S-TE, C-CIP-CN-TE, AMP-C-CN-TE C-CN-S-TE, AMP-CTX-EFT-TE	0.4
5	8(10.7%)	AMP-CTX-C-SXT-TE AMP-CN-S-SXT-TE AMP-C-S-SXT-TE AMP-C-CN-SXT-TE	31 (13.5%)	AMP-C-CIP-SXT-TE, AMP-C-S-SXT-TE, AMP-CIP-S-SXT-TE AMP-C-CIP-S-TE, CTX-CIP-S-SXT-TE, AMP-C-CN-S-SXT AMP-C-CT-CN-TE, AMP-C-CN-SXT-TE, AMP-CTX-CIP-S-TE AMP-CTX-EFT-C-TE	0.5

Table 4 (continue)

No. of antimicrobial	*Salmonella* isolates (n=75)		*E. coli* isolates (n=230)		MAR Index
	No. of isolates	MDR profile	No. of isolates	MDR profile	
6	2 (2.7%)	AMP-C-CIP-S-SXT-TE AMP-C-CN-S-SXT-TE	53 (23%)	AMP-CTX-EFT-C-CN-TE, AMP-C-CIP-S-SXT-TE AMP-CTX-EFT-CIP-S-TE, AMP-C-CIP-S-SXT-TE C-CIP-CN-S-SXT-TE, AMP-CIP-CN-S-SXT-TE AMP-C-CIP-CN-S-TE, AMP-C-CN-S-SXT-TE AMP-C-CIP-CN-SXT-TE, CTX-EFT-CIP-S-SXT-TE AMP-CTX-CIP-S-SXT-TE, AMP-CTX-C-CIP-CN-TE	0.6
7	N/A		25 (10.9%)	AMP-C-CIP-CN-S-SXT-TE, AMP-CTX-EFT-CIP-S-SXT-TE AMP-CTX-EFT-C-S-SXT-TE, AMP-CTX-C-CIP-S-SXT-TE AMP-CTX-C-CIP-CN-SXT-TE, AMP-CTX-C-CN-S-SXT-TE AMP-CTX-C-CIP-S-SXT-TE, AMP=CTX-EFT-CIP-S-SXT-TE	0.7
8	5 (6.7%)	AMP-CTX-EFT-C-CN-S-SXT-TE	8 (3.5%)	AMP-CTX-EFT-C-CIP-S=SXT-TE, AMP-CTX-EFT-CIP-CN-S-SXT-TE, AMP=EFT-C-CIP-CN-S-SXT-TE, AMP-CTX-EFT-C-CIP-S-SXT-TE, AMP-CTX-EFT-C-CIP-CN-S-TE, AMP-CTX-EFT-C-CIP-CN-S-SXT-TE	0.8
9	N/A		17 (7.4%)	AMP-CTX-EFT-C-CIP-CN-S-SXT-TE	0.9
10	N/A		1 (0.4%)	AMP-CTX-EFT-C-CIP-CT-CN-S-SXT-TE	1.0

CONCLUSION

In conclusion, the significant prevalence of multi-drug resistant *Salmonella* spp. and *E. coli* identified in animal food products from central Peninsular Malaysia highlights the urgent need for effective monitoring and surveillance of antimicrobial use and resistance within the food system. These findings will aid in conducting thorough assessments of foodborne AMR risks and guide the creation of effective prevention and mitigation strategies to protect public health. Ongoing vigilance and a coordinated, multi-stakeholder approach are vital for addressing this complex issue and maintaining the long-term effectiveness of antimicrobial agents.

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