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Review Paper

Review of antimicrobial-resistant ESKAPEE pathogens in food sources and their relevance to the One Health approach

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ABSTRACT

Antimicrobial resistance (AMR) is a growing concern globally, particularly with ESKAPEE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*) which have developed multidrug resistance. While traditionally associated with healthcare settings, these pathogens are increasingly being found in food sources, particularly meat products, and agricultural environments. This review explores the mechanisms by which ESKAPEE pathogens acquire antimicrobial resistance, their occurrence in meat and other food products, and the implications for food safety. It also discusses the environmental reservoirs, including water sources, and the challenges posed by the spread of these pathogens within the meat production and processing chains. Surveillance programs monitoring the prevalence of AMR in the food industry, especially in relation to meat, are outlined, highlighting the need for improved detection and control measures. The review emphasizes the importance of a One Health approach, recognizing the interconnectedness of human, animal, and environmental health. Addressing AMR in meat and food production requires coordinated efforts across sectors, including public awareness campaigns and enhanced food safety protocols, to limit the spread of resistant ESKAPEE strains.

1. Introduction

Antimicrobial resistance (AMR) is a natural evolutionary process by which microorganisms adapt to antimicrobial agents (*Fair & Tor*, 2014), but it has become a major global health threat, often referred to as a silent pandemic, with over 700,000 deaths annually (*O'Neill*, 2016). In 2019, AMR was directly responsible for approximately 1.27 million deaths and contributed to nearly 4.95 million additional deaths (*WHO*, 2018). Limited treatment options for infections caused by resistant bacteria further exacerbate the issue (*Magiorakos et al*., 2012). The World Health Organization (WHO) has identified priority pathogens, particularly multidrug-resistant (MDR) Gram-negative bacteria from the ESKAPEE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp., and *Escherichia coli*), as critical threats (*Tacconelli*, 2020; *WHO*, 2023). While multi-drug resistant (MDR) infections

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are commonly associated with hospitals, resistant bacteria are increasingly found in animals, food, and aquatic environments (*Lepuschitz et al*., 2019; *Wyres & Holt*, 2018). Antibiotic use in agriculture promotes resistance development, which can be transmitted to humans through direct contact, contaminated food, or the environment (*Argudín et al*., 2017). Resistance often spreads via horizontal gene transfer through mobile genetic elements (*Gajic*, 2023). Extended-spectrum β-lactamase (ESBL)- and carbapenemase-producing bacteria, once limited to hospitals, are now also detected in animals (*Dahms et al*., 2015; *Michael et al*., 2015), and the emergence of colistin-resistant *Enterobacteriaceae* carrying *mcr* genes raises further concerns (*Liu et al*., 2016).

2. Understanding antimicrobial resistance pathways in ESKAPEE bacteria

Antimicrobial resistance genes can be located on bacterial transposons, plasmids, or the chromosome (*Giedraitienė et al*., 2011). These genes, especially when carried on mobile genetic elements (MGEs), can be acquired by bacteria through horizontal gene transfer or arise through genetic mutations, enabling pathogens, particularly those from the ESKAPEE group, to develop various mechanisms of resistance. ESKAPEE pathogens have developed multiple mechanisms of antibiotic resistance. These mechanisms include: drug inactivation, modification of antibiotic binding sites, active drug efflux via efflux pumps, reduced drug uptake and biofilm production (*Oyenuga et al*., 2024).

2.1 Drug inactivation

Numerous bacterial enzymes are capable of permanently altering or neutralizing the effects of antibiotics. These include aminoglycoside-modifying enzymes, β-lactamases and chloramphenicol acetyltransferases. Among them, β-lactamases are particularly well-studied and widespread. They function by hydrolysing the β-lactam ring, a core structural component of all β-lactam antibiotics, thereby rendering carbapenems, cephalosporins, penicillins and monobactams ineffective (*Reygaert*, 2018).

2.2 Modification of antibiotic binding sites

Many resistant bacteria evade antimicrobials by modifying their target sites. In multi-drug resistant *S. aureus* (MRSA), mutations in *mecA* or *mecC* lead to production of altered penicillin-binding proteins (PBPs), such as PBP2a, which have low affinity for β-lactam antibiotics, allowing cell wall synthesis to continue despite treatment (*Tang et al*., 2014; *Oniciuc et al*., 2017; *Abebe & Birhanu*, 2023; *Santajit & Indrawattana*, 2016). Similarly, *E. faecium* and *Enterococcus faecalis* resist glycopeptides like vancomycin and teicoplanin by substituting D-Ala-D-Ala with D-Ala-D-Lac or D-Ala-D-Ser in peptidoglycan precursors, a change mediated by resistance genes such as *VanA*, *VanB*, and others, which prevent antibiotic binding (*Giedraitienė et al*., 2011).

2.3 Active drug efflux via efflux pumps

Efflux pumps are membrane proteins that help bacteria expel antimicrobial agents, lowering intracellular antibiotic levels and reducing drug efficacy (*Santajit & Indrawattana*, 2016). These pumps play a key role in multidrug resistance by exporting a wide range of antibiotics. Five major efflux pump superfamilies are recognized: ABC (ATP-dependent), and four proton sodium gradient-driven families, SMR, MFS, RND, and MATE. A sixth group, the PACE family, has also been identified (*Du et al*., 2018). In *P. aeruginosa*, systems like MexAB-OprM and MexCD-OprJ contribute to resistance against fluoroquinolones, aminoglycosides, and carbapenems (*Jamal et al*., 2023).

2.4 Reduced drug uptake

Bacterial antibiotic susceptibility depends on the balance between drug entry and efflux. One resistance mechanism involves reduced membrane permeability, limiting drug uptake (*Uddin et al*., 2021; *Santajit & Indrawattana*, 2016). In Gram-negative ESKAPEE bacteria, porins facilitate antibiotic entry, but mutations or loss of porins, such as in *K. pneumoniae*, can block drug entry and confer resistance to β-lactams (*Santajit & Indrawattana*, 2016).

2.5 Biofilm production

Biofilm formation involves three main stages: initial bacterial adhesion to a surface, followed by growth and secretion of extracellular polymeric substances (EPS) that form a protective matrix, and finally, detachment. Detachment can occur actively

via quorum sensing and enzymatic matrix degradation, or passively due to physical forces like shear stress or human handling (*Laverty et al*., 2014).

3. Presence of ESKAPEE pathogens in the food chain

Holman et al. (2021) reported that *E. faecalis* and *E. faecium* were isolated from swabs and ground beef in a beef processing facility, with *E. faecalis* being more prevalent and present at all sampling points. Most *E. faecalis* isolates exhibited high resistance to lincomycin (97.4%) and quinupristin-dalfopristin (93.2%), along with varying levels of resistance to tetracycline, erythromycin, tylosin, and ciprofloxacin, while *E. faecium* isolates showed similar resistance profiles, except for ciprofloxacin, to which they remained susceptible. Notably, only one *Enterococcus* isolate showed full susceptibility to all 16 tested antimicrobials, with no resistance recorded to linezolid, penicillin, or vancomycin in any isolate. Complementary findings were reported in retail food studies across Europe. *Pesavento et al.* (2014) detected *E. faecium* in 35.5% of raw meat (beef, poultry, pork) and 44.9% of ready-to-eat products (cheese, salads, ham) in Italy, with resistance most commonly found to erythromycin (22.1%), tetracyclines (16.4%), gentamicin (13.6%), and ciprofloxacin (10.7%). In Turkey, *Sanlibaba et al.* (2018) found *E. faecium* in 61.9% of pre-packaged chicken meat samples, with dominant resistance to rifampicin (81.7%) and significant rates for ampicillin (73.3%), erythromycin (45%), and ciprofloxacin (31.7%). Similarly, a Polish study revealed that 31.6% of fermented milk products were contaminated with *E. faecium*, showing resistance to streptomycin (29.7%), erythromycin (14.9%), and tetracyclines (10.9%) (*Chajęcka-Wierzchowska et al*., 2020). Together, these findings underscore the widespread presence of multidrug-resistant *Enterococcus* spp. across diverse food sources in Europe.

Contamination of meat and meat products with *S. aureus* is widespread. *Wu et al.* (2018) found it in 35% of 1850 retail meat samples, with high resistance rates to ampicillin (85.4%), penicillin (84.6%), erythromycin (52.7%), and tetracycline (49.3%). Similarly, raw chicken meat contained *S. aureus* strains fully resistant to cefpodoxime and cloxacillin (100%), with notable resistance to ceftazidime and piperacillin/tazobactam (92.5%), clindamycin (72.5%), and methicillin (57.5%) (*Herve & Kumar*, 2017). *K. pneumoniae* was detected in 46.7% of raw calf and chicken meat samples in Turkey, with all isolates resistant to ampicillin and amoxicillin, and 29% resistant to aztreonam (*Gundogan et al.,* 2011). In another study, fresh raw poultry showed the highest contamination (13.8%), and isolates displayed resistance to ampicillin (92.3%), tetracycline (31.3%), and trimethoprim-sulfamethoxazole (18.2%) (*Liu et al.,* 2016). *A. baumannii* was found in 20% of raw meat samples, exhibiting high resistance to gentamicin (87.2%), tetracycline (79.5%), erythromycin (74.4%), and ciprofloxacin (59%) (*Askari et al*., 2020). *P. aeruginosa* was isolated from 29 out of 370 samples of raw, frozen, and processed beef products, with most isolates resistant to ampicillin (89.6%), penicillin (86.2%), and tetracycline (82.7%) (*Rezaloo et al*., 2022). In Egypt, 139 *Enterobacterales* strains isolated from RTE foods including meats showed general susceptibility, though resistance to ampicillin, third-generation cephalosporins, and carbapenems was noted. *Edris et al*., (2023) reported *Enterobacter* spp. in 274 meat-related products, with *E. cloacae*, *E. hormaechei*, and *E. kobei* resistant to β-lactams, cephalosporins, and, in some cases, glycopeptides; several strains were MDR or even extensively drug resistant (XDR). *Menck-Costa et al*. (2023) found ESBL-producing *E. coli* in 37% of 450 meat samples, especially in chicken (109/150), with additional resistance to tetracycline (51%), ciprofloxacin (46%), and fosfomycin (38%). Notably, 45% of these *E. coli* strains were MDR, though all were susceptible to imipenem. Another study detected *E. coli* in 33.8% of milk and dairy products, with 88.1% of tested strains resistant to at least three antimicrobials (*Sarba et al.,* 2023). An *E. coli* O157 strain was also identified in milk (0.2% of *E. coli* isolates) (*Sarba et al.,* 2023). Although not meat-related, *E. coli* from vegetables also showed resistance to penicillin (100%) and other antibiotics (*Datta et al*., 2024).

4. The role of the One Health concept in combating AMR

The complexity of AMR at the human-animal-environment interface requires an integrated, multidisciplinary approach. The One Health concept, recognizing the interconnectedness of human, animal, and environmental health, is key to addressing antibiotic resistance gene spread. These genes move through hospitals, farms, and water sources, acting as “reactors” for resistance (*Gajić*, 2023). However, the One Health approach has not been widely imple-

mented. Strengthening the understanding of genetic links among MDR organisms can enhance national strategies. While clinical resistance is well-documented, data on environmental resistance is lacking. National and international strategies emphasize prudent antibiotic use, infection prevention, surveillance, and scientific knowledge. WHO's GLASS initiative recommends tools like whole-genome sequencing (WGS) for understanding resistance patterns and transmission routes (*WHO*, 2022). "One Health genomics," using metagenomics and advanced sequencing, helps map AMR, identify resistance hotspots, and clarify genetic relationships between MDR bacteria, guiding targeted interventions.

4.1 AMR: a public health issue in Serbia

AMR poses a significant public health issue in Serbia. In 2019, approximately 1,800 deaths were directly linked to AMR, with 7,100 deaths associated with resistant infections, surpassing mortality from diseases like diabetes and chronic respiratory conditions. Serbia ranks 68th globally in AMR-associated mortality per 100,000 people. The main pathogens contributing to the AMR burden are *E. coli* (1,800 deaths), *S. aureus* (1,200), *K. pneumoniae* (986), *P. aeruginosa* (617), and *Streptococcus pneumoniae* (493), which cause bloodstream, intra-abdominal, respiratory, and urinary tract infections (*Institute for Health Metrics and Evaluation*, 2019).

5. Conclusion

Effectively addressing AMR requires full implementation of the One Health approach, which promotes global collaboration, innovation, long-term investment, and strong governance. The rise of multidrug-resistant ESKAPEE pathogens, including those with resistance to last-resort antibiotics, poses a serious clinical challenge. Overuse of antibiotics in healthcare and food production worsens the problem, increasing pressure on health systems. Reducing AMR depends on restricting antibiotics to treatment, avoiding their use for prophylaxis or growth promotion, and enforcing strict regulation, monitoring, and reporting to limit environmental contamination. A coordinated, cross-sectoral effort is essential to combat AMR.

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