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# Some Multidrug Resistant Pathogens Isolated From Food of Animal origin Reham, M. Abdel- Wahhab\*; Rania, A. Abdel Kader\*; Wafaa, F. Ahmed\*; Hend, A. Mahmoud\*\* and Soad, H. El-Sheikh\*

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# **Review Article**

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#### Abstract

The food of animal origin such as Red meat, poultry meat, milk and their products have a great importance in human nutrition due to their contents of essential amino acids, But the danger lies in their content of pathogenic microorganisms especially if these organisms are multidrug resistant, the problem which arises from the overuse of antimicrobial drugs that happen during animal growth, either as a prophylactic or therapeutic treatment. It has been recorded that at least twenty five percent of the foodborne isolates expose drug resistance to one or more groups of antimicrobials. An analysis conducted on the antibiotic resistance of pathogens obtained from different food products reveals that the average frequency of their existence in food is  $\geq 11\%$ . Meanwhile, multidrug resistance is directed to  $\beta$ -lactam antibiotics. Some food poisoning microbes were discussed, with referring to the most important mechanisms of development of drug resistance

Keywords: Antibiotic, resistance, food poisoning, bacteria

#### Introduction

Abundant pathogens may earn access in to food of animal origin from various sources and induce varieties of food borne diseases either via the microbe itself or via its toxins. Relied on the type of disease induced, these illnesses are classified as food infection, food intoxication and food toxi-infection. It's a major issue, but the most prominent is when the causative organism is resistant to antibiotics, shockingly on the off chance that it is multidrug resistant one. Multidrug resistance refers to bacteria's ability to resist three or more classes of antibiotics that target various microorganisms Nikaido, (2009). The issue in the meat and dairy industry; is the broad administration of antimicrobial agents to evade, control, or treat contamination in animals and to render as growth enhancers. The uncontrolled administration of these drugs to cattle may develop resistance versus bacterial pathogens **Sharma** *et al.*, (2011). The exchange of genes responsible for antimicrobial resistance from one bacterium to another is the result of this behavior. Resistant bacteria eventually end up in the food chain Nisha, (2008) and Sharma *et al.*, (2011). Resistant foodborne microbes have gotten to be a food safety defiance that requests a one-health concern based arrangement. Making it worse; several previously treatable bacterial contaminations have, over time, developed resistance to certain antimicrobial agents **Nyenje and Ndip**, (2013). Each day, the number of resistant microbes to all known antibiotics is in boost, leading to the expectation that by 2050, "humanity may return to an era without antibiotics" **Durão** *et al.*, (2018). The point of this article is to crystallize the problem of antibiotic-resistant organisms in foods of animal origin

# Foodborne Pathogen Transmission

Meat from animals or birds can infect humans with foodborne illnesses in a number of ways. Numerous domestic and wild animals, particularly those raised for human consumption, have had their digestive tracts colonized by foodborne pathogens such as Salmonella, Campylobacter, and pathogenic E. coli Zhao et al. (2001). These bacteria have the ability to contaminate food at many different phases of the food supply chain, such as handling or preparation, processing, distribution, retail marketing, and production. Eating foods derived from animals increases the chance of contracting foodborne illnesses significantly, according to a number of epidemiological studies Petersen and James (1998).

It is believed that poultry is a significant asymptomatic carrier of several infectious illnesses. According to Mir et al. (2015), one of the main reasons for the cross-contamination of the carcass with Salmonella and Campylobacter is the removal of the gastrointestinal tract during slaughter. However, the predominant route of foodborne disease transmission from poultry to humans is through the ingestion of contaminated meat. Meat processing, storage, or slaughtering is the possible situations during which contamination happens Hafez, (1999). According to an African study, selling carcasses without cold storage or protection from dust and flies raised the risk of microbial contamination of meat, especially from foodborne pathogens like Salmonella and Campylobacter Kagambèga et al., (2018). Poor hygienic practices of poultry meat handlers also contributed to this risk. Furthermore, elements such as unsuitable storage conditions; such as storing meat for an extended length of time also encourage microbial growth in meat

and render it unfit for human consumption Taulo et al., (2008).

The dairy cow mammary gland can transmit pathogens directly, or indirectly, via body secretions that can fall, inflate, or splash into milk. Handlers play a crucial part in addition to coughing, sneezing, and talking when milking or handling milk on a farm, contaminated hands can spread illnesses like typhoid, infantile diarrhea, diphtheria, scarlet fever, and septic sore throat. Moreover, the environment pathogens may also be introduced into milk products by the dairy farm environment at various phases of production and processing. Contaminated water, feed, and milk-handling equipment, together with other unsanitary conditions at the farm and factory, may have a substantial impact on pathogens and microorganisms that cause spoiling in milk Almashhadany et al., (2022).

# Some resistant pathogens isolated from food of animal origin

# 1- Staphylococcus aureus.

In humans and other animal species, S. aureus is an opportunistic pathogen. The pathogen is the third leading cause of food-related illness worldwide and is primarily linked to food poisoning Sasidharan *et al.*, (2011). According to Hennekinne et al., (2012) strains of coagulase -positive staphylococci, primarily S. aureus, are responsible for a range of infectious disorders; dermatitis, meningitis, osteomyelitis, pneumonia in humans as well as mastitis in cattle, and bumble foot illness in poultry. Since a few decades ago, S. aureus has been linked to 25% of all foodborne diseases in the USA. Due to ongoing antibiotic misuse; methicillinresistant S. aureus strains have emerged realization as one of the most grave nosocomial bacteria that induce food poisoning. Despite the low prevalence of MRSA in food, the problem stems from the strains' multidrug resistance, which makes treating infections difficult Sciezynska et al., (2012). Multiple antimicrobial resistance patterns have been shown to be regularly observed in S. aureus Enright, (2003). This could be because the overuse of antibiotics has resulted in the emergence of certain antibiotic resistances, making antibiotic therapy useless. In soil, water, plants, and animals, resistant microorganisms can be found. Both humans and animals come into contact with the resistant bacteria that exist in their surroundings. According to estimates, humans consume almost the same amount of antimicrobial drugs **Farzana** *et al.*, (2004)

Because resistant bacterial strains are constantly circulating in the environment and can contaminate food and water, antimicrobial resistance is a serious public health concern in many countries Normanno et al., (2007). In the past, S. aureus strains were almost always penicillinasesensitive to semi-synthetic resistant  $\beta$ -lactam antibiotics, such as methicillin and oxacillin, which are the most widely utilized class of antibiotics for skin infections. The strains were referred to as "methicillinresistant Staphylococcus aureus" (MRSA) which suggested that they were resistant to all  $\beta$ -lactam antibiotics, such as penicillins and cephalosporins.

# 2- Salmonella spp.

There are 2610 distinct serotypes of Salmonella enterica subspecies enterica; the most wellknown serotypes include *Typhi, Paratyphi, Enteriditis, Typhimurium, and Choleraesuis* **SuandChiu, (2007).** According to **Bronze and Greenfield (2005),** the three surface antigens that define the serotypes are the polysaccharide "Vi" antigen (present in the Typhi and Paratyphi serotypes), the oligosaccharide "O" antigen, and the flagellar "H" antigen. Salmonellosis is usually linked with consuming food products contaminated with Salmonella species, especially eggs, poultry meat products.

Contact with diseased pets or inadequate hand washing are two ways that contamination might happen Munck et al., (2020). Salmonella spreads primarily through food handlers of "meat processing" and "ready-to-eat" foods Ehuwa et al., (2021). The key origins of the disease's transmission are thought to be the sale of contaminated food items and goods made with inferior quality FAO/WHO, (2004); Bettridge et al., (2014).Concern over antimicrobial tolerance in relation to human and animal salmonellosis is developing Suetal., (2004). As reported, Salmonella strains resistant to antibiotics are prevalent in most regions of the world and have boosted obviously in the last decade WHO, (2018).

The frequency of animal-to-human transmission is rising quickly. According to Shrestha et al. (2017), the problem of antimicrobial drug resistance is particularly severe in underdeveloped nations. Salmonella strains that are obtained from food, particularly poultry products Ehuwa et al., (2021); Gargano et al.,(2021); Raji et al., (2021), are the most common source of multidrug resistance. One of the main worries is that these bacteria have the capacity to spread their resistance genes to bacterial pathogens that affect humans Musawa et al., (2021). Notably, multidrug resistant Salmonella serovars obtained from chicken embryos in Henan province, China, gave an alarm for the immediate lowering in the usage of antimicrobial therapy in chicken hatcheries. Furthermore, different patterns found by pulsed-field gel electrophoresis among the Salmonella serovars suggest the existence of many sources of contamination Xu et al., (2021).

The study conducted by Samy et al. (2022) investigated the prevalence of multiple drug resistance (MDR) Salmonella in Egyptian food originating from animals. The results indicated that the bacteria were resistant to 100% erythromycin, 57% ampicillin, 50% cefradine, and 50% amoxicillin in milk and milk products. Additionally, the study found that a high incidence of multiple antibiotic resistances was found in poultry sources, where the highest was 100% versus oxytetracycline, persuaded by amoxicillin 73.3% and ampicillin 66.6%. On the other side, in meat and meat products, the highest incidence of Salmonella resistance was against cefradine, 53.7%, 50% ampicillin, and 31.4% oxytetracycline and amoxicillin. Food safety agencies need to consider these significant percentages in order to implement the required actions.

# 3- Escherichia coli spp.

*Escherichia coli* are commensal bacteria that present among large variety of hosts. They commonly exist in the atmosphere and are informed to be an indicator of water and food fecal pollution. *E. coli* from other species in the ecosystem may evolve, keep, and transfer resistance genes because of its ubiquitous existence and its attitude as a commensal or pathogenic organism in human and animal.

E. coli-related foodborne infections are a sig-

nificant global public health concern that is primarily spread by eating contaminated food. The existence of *E. coli* in poultry and beef meat has important public health consequences **Sousa**, (2008).

*E. coli* isolated from food of animal origin recorded high resistance to ampicillin, amoxycillin-clavulanic acid, trimethoprim/ sulphamethoxazole, tetracycline, and streptomycin Ahmed and Shimamoto (2013); Ahmed and Shimamoto (2015); Moawad *et al.* (2017).

#### 4- Bacillus cereus

В. cereus is Gram-positive, aerobic-tofacultative, spore-forming, motile rod-shaped that is broadly found in food and the environment. It generates the enzyme lecithinase, spores, and enterotoxins. According to Hwang and park (2015), it is mostly found in soil, milk, grains, spices, and other dried foods. B. cereus is one of the common bacteria found in the environment and the cause of food poisoning Rasko et al., (2005). Additionally, it has been discovered that *B. cereus* is extensively distributed in human intestine, food, and soil Bottone, (2010). In seventy seven outbreaks and 17.1% of cases involving bacterial toxins, B. cereus has been identified as the causal agent of food-borne outbreaks, according to the European Food Safety Authority's report EF-SA, (2006).

According to **Osama** *et al.*, (2020) study, 100% of *B. cereus* isolated from milk and milk products found to be resistant to ampicillin, amoxicillin and colistin persuaded by 83.01% resistant to ampicillin-sulbactum, 67.9% resistant to streptomycin, then resistant to spiramycin, lincomysin, tetracyclin, and erythromycin by 45.2, 35.8, 22.6, and 5.6% respectively. These data also harmonized with **Kim** *et al.* (2011) who found that all studied *B. cereus* strains were resistant to  $\beta$ - lactam group comprising ampicillin, amoxicillin and penicillin while were susceptible to vancomycin, tetracycline, gentamycin, and ciprofloxacin.

In addition, according to Abd-El Tawab *et al.* (2020), *B. cereus* isolated from meat and meat products exposed a high level of resistance to penicillin-G (92.2%), methicillin (90.2%), followed by ampicillin, oxytetracycline, sulfatrimethoprim, and cefotaxime as 88.2, 82.3,

80.4, and 54.9% respectively. In the meantime, the strains exhibited high susceptibilities to gentamycin (82.3%), ciprofloxacin (74.5%), meropenem (72.5%), and florphenicol (62.8%), on the other manner, intermediate susceptibilities were shown versus streptomycin (62.8%), erythromycin (58.8%), and neomycin (56.9%).

### 5- Listeria monocytogenes

Listeria monocytogenes is a rod-shaped coccobacillus that is facultatively anaerobic, Grampositive, and does not generate spores. Its normal dimensions are 0.5 to 2µm in length and 0.5µm in diameter Acha and Szyfres, (2003). L. monocytogenes can proliferate at temperatures between 1 and 45°C and can thrive at a wide pH range (4.3 to 9.6) Shamloo et al., (2019). Globally, foodborne illness outbreaks and high hospitalization rates are linked to the foodborne bacterium L. monocytogenes Osman et al., (2020).

Various foods and dairy products contain L. monocytogenes, which is the cause of listeriosis in people and animals Skowron et al., (2019). The bacteria have also been linked to recent outbreaks and isolated cases of listeriosis Kim et al., (2018). It is also thought to be a possible source of contamination for cold and marine food products Vinothkumar et al., (2013) as well as unpasteurized dairy products. According to research by Abdeen et al., (2021) the antibiogram profile of L. monocytogenes revealed a high sensitivity to amoxicillinclavulanic acid (76.4%), cefotaxime (70.5%), intermediate sensitivity to erythromycin, amoxicillin (64.6%), then gentamicin and vancomycin (58.7%). On the other hand, there was significant resistance to trimethoprim-(76.4%), sulfamethoxazole azithromycin (41.2%), levofloxacin (41.2%), doxycycline (64.6%), chloramphenicol (70.5%), and oxytetracycline (76.4%). L. monocytogenes strains gathered from 4 distinct sources; fish fillet, minced meat, raw milk, and sausage had generally comparable antimicrobial characteristics, moreover, all of the isolated L. monocytogenes were multidrug-resistant Abdeen et al., (2021).

#### 6- Brucella spp.

Brucella is a Gram negative coccobacillus belonged to the family Brucellaceae. The currently recognized species involve B. abortus, B. melitensis, B. ovis, B. suis, B. ceti, B. canis, B. neotomae, B. microti, B. pinnipedialis, and B. inopinata. Humans contract the disease primarily by direct contact with the discharges of infected animals, consumption of unpasteurized milk or dairy products, and undercooked meat or meat products Nejad et al., (2020).

According to Hayoun *et al.* (2020), there are more ways that Brucella can infect humans, including cutaneous abrasions, conjunctival inoculations, and aerosol inhalation. Human brucellosis symptoms are quite diverse and non -specific. The illness can produce fever, but its symptoms are similar to those of other feverish illnesses, leading to incorrect diagnoses and inadequate treatment. Chronic brucellosis can result from Brucella infection in the genitourinary system, joints, parenchymatous organs, and bone. After therapy, many relapses were documented Wang *et al.*, (2020).

Brucella has maintained, to some degree, its sensitivity to rifampicin, aminoglycosides (gentamicin), fluoroquinolones (levofloxacin and ciprofloxacin), new glycylcycline (tigecycline), and doxycycline, according to **Wareth** *et al.* (2021) Rifampicin remains the primary common treatment choice for human brucellosis, usually in conjunction with other drugs.

Otherwise, a different study conducted by **Khanetal**, **(2019)**, 75.2%, 66.7%, and 4.8% of animal-origin *B. melitensis* strains tested positive were resistant to ciprofloxacin, rifampicin, and streptomycin, respectively. According to a prior investigation, 277 (64%) of the 355 *B. melitensis* isolates with human origin in Egypt were resistant to rifampicin **Abdel-Maksoud** *et al.*, **(2012).** 

#### Mechanisms of development of drug resistance

Anti-Microbial Resistance (AMR) takes place when bacteria, fungi, viruses, and parasites are presented for a long time to sub-therapeutic doses of drugs that modify the ecology of microorganism. In order to combat with the residues exhibit in their environment, microorganisms may gain genetic components that permit them to overcome these compounds and survive. In some cases, the use of poor quality drug, fraud products, faulty product, and subtherapeutic dosage can boost the emergence of microbial resistance. Another relevant component for up-growth of AMR is the inconvenient disposal of waste output in the agricultural production, pharmaceutical and wastewater treatment plants as they can be disseminated via the environment **FAO**, (2019).

The effectiveness of antimicrobials is compromised by an increasing number of antibiotic resistant pathogens.

Resistance can be depicted in two ways:-

A). **Intrinsic resistance** whereby microorganisms naturally have not target sites for the antimicrobials so the antimicrobials cannot affect them.

B). Acquired resistance whereby a naturally susceptible microorganism earns mechanism to not be influenced by the antimicrobial. Mechanisms of acquired resistance comprise; the existence of an enzyme that inactivates the antimicrobial agent, post-transcriptional or posttranslation modification of the antimicrobial agent's object, minimized uptake to the antimicrobial drug as well as the active efflux of the antimicrobial agent.

The intrinsic and acquired resistances are summarized by **Mancilla-Becerra** *et al.*, (2019), in figure 1.



Figure (1). Schematic representation of the mechanisms of MDR gaining in bacteria.

The mechanisms of action and resistance to antibiotic groups were tabulated in table (1) according to **Dowling** *et al.*, (2017).

Antimicrobial Family	Mechanism of Action	Resistance Mechanism
Beta-lactam antibiotics	Prohibits cell wall production.	Beta-lactamase production
Cephalosporins	Binds enzymes (PBPs) that help	primarily- bla genes. Changes
Beta-lactamase inhibitors	form peptidoglycans.	cell wall protein enzymes to
	Inhibits/binds to beta-lactamase	prevent binding to PBPs.
	enzymes.	Cephalosporinases Extended-
		spectrum beta lactamases
		(ESBLs). Class A-D.
Aminoglycosides	rRNA- binds to 30S subunit,	Phosphorylation, adenylation and
	causing genetic code misread.	acetylation of aminoglycoside
	Inhibits protein production.	stops them binding.
	Effect on cell membrane	
	permeability	
Fluoroquinolone	Interrupts DNA breakage-	Target modification of DNA
	reunion step by binding DNA-	gyrase (gyrA and gyrB).
	gyrase or topoisomerase II and	Decreased permeability- outer
	topoisomerase IV.	membrane porins mutations
		(ompF). Efflux pumps.
Folate pathway inhibitors.	Purine synthesis for DNA.	Chromosomal mutations but
	Interferes folic synthesis.	more commonly plasmid and
		integron-mediated resistance.
		Pathway blocked by resistant
		dihydrofolatereductase (dfr gene).
Tetracycline	rRNA- binds to 30S subunit and	Inducible efflux E. coli etc. (tetA,
	interferes with amino acid	tetB, tetC). Binding site changes
	transfer. Prevents protein	(tetO, tetM genes). Activate V
	production.	Go to Setting

Table (1). Mechanism of action and resistance of prevalent used antimicrobial drugs.

# Techniques for detection of antimicrobial resistance (AMR):

Although novel time-saving technologies have been introduced to gain antimicrobial resistance data, the classic, conventional technologies are still being used. These fundamentally comprise culture-based and molecular-based approaches. More recently, microscopy-relied and spectrometry-relied approaches have also been integrated in the tools for developing diagnostics.

# **1-** Phenotypic Methods

A culture-based method relies on the phenotypic resistance detection by testing the bacterial growth in the existence of antibiotics, and can be categorized in two classes:

**Manual tests** were expressed as disk diffusion, minimum inhibitory concentration (MIC) determination and The E-test® or gradient test.

#### Disk diffusion method:

It is the process of an antibiotic diffusing over agar in a paper disc, with an inhibition zone forming around the disc. The results are categorized as resistant, intermediate, or susceptible using a qualitative method. It is a simple, useful technique that works well for bacteria that grow quickly. However, there are several limitations, such as the usage of antibiotics that don't diffuse well in agar and challenging interpretation for anaerobic and fastidious microorganisms **Sejas** *et al.*, (2003).



Figure (2). Disk diffusion method

#### **MIC detection**

When testing in broth or agar, the goal of MIC determination is to measure the minimum antibiotic concentration that prevents detectable bacterial growth. Agar or broths with varying antibiotic concentrations are inoculated with solutions containing a specific number of bac-



Figure (3). MIC on agar

teria. It is checked for microbial growth at the conclusion of the incubation time. It is an easy, low-cost procedure that doesn't need any specialist tools. However, because it uses a semiquantitative approach, a precise MIC value might not be determined **Wiegand** *et al.*, (2008).



Figure (4). MIC detection on broth by microtitre plate

**E-test® or gradient test:** This technique combines the last two approaches that were discussed. It calculates MIC but has disk diffusion -like processing. A rectangular device is set up on an agar plate with the interpretation scale and the antimicrobial concentration gradient on opposite sides. Despite the fact that the E-test® has the same execution time limitations as the two tests mentioned earlier, it offers a simpler method of directly quantifying the susceptibility of microorganisms, particularly those that are hard to culture (like *Mycobacterium bovis* and *Haemophilus influenzae*) or even anaerobes **Jorgensen**, (1997). It also has an immobilized antimicrobial gradient indicated on the ruler



Figure (5). E-test® or gradient test for detection of antimicrobial resistance

Automated commercial platforms (VITEK® 2 COMPACT, Sensititre<sup>TM</sup> ARIS<sup>TM</sup> 2X, and Alfred 60AST system) use some of the aforementioned methods.

Automated MIC panel inoculation is the first step of an automated testing system. Computer -assisted incubation with reading, interpretation, and reporting features that eliminate the need for manual intervention follows **Evangelista and Karlowsky (2017)**.

# 2- Molecular Techniques

Molecular-based techniques have features over phenotypic assessments in detecting antibiotic resistance genes (ARGs), such as multiplex PCR, giving more precise identification and detection. These techniques display a viable alternative in certain taxonomic units where susceptibility breakpoints have not been realized. Non-purified polymicrobial samples can also be used in molecular-relied approaches, which can direct to faster response times to newly added resistance factors Vasala *et al.*, (2020).

However, due to their limited sensitivity and breadth, molecular-based approaches have limitations, including the inability to estimate MICs and the potential to overlook some ARGs. Furthermore, it can be expensive to create molecular-based assays that can identify the wide variety of AMR genes Fluit *et al.*, (2001). However, utilizing amplification and nucleic acid hybridization techniques to improve ARG expression and detection, molecular-based treatments are constantly being refined. In general, ARG may be detected quickly and sensitively using molecular-based methods Sundsfjord *et al.*, (2004).

#### **3- Innovative Approaches**

3-1. Mass Spectrometry-Based Methods

Antibiotic resistance can now be found via matrix-assisted laser desorption/ionization-timeof-flight mass spectrometry (MALDI-TOF MS), a quick and accurate technique for identifying microbes in clinical isolates and clinical samples. Three techniques are available for detecting AMR with MALDI-TOF MS: (1) measuring bacterial growth while exposed to an antibiotic, (2) evaluating antibiotic changes due to enzyme activity, and (3) identifying AMR by looking at pathogen peak patterns **Oviaño and Bou, (2018)**.

3-2. Bioinformatics Approach for Detection of AMR Determinants and Databases

Bioinformatics helps us assess the vast amount

of data gathered to choose specific approaches that are situation-specific and improve treatment, monitoring, and preventive strategies for infections caused by bacteria resistant to antibiotics **Seoane and Bou**, (2021).

3-3. Microfluidics, Biosensors and Nanotechnology

Micro-Electro-Mechanical Systems (MEMS) biosensors were utilized by Koydemir et al. (2011) to effectively identify MRSA. In parallel, Xuetal. (2018) reported the development of a very sensitive electrochemical biosensor for identifying the *mecA* gene in MRSA strains.

# Conclusion

In this review, we highlight the problem of antimicrobial resistance in food of animal origin With clarification of different mechanisms of antimicrobial resistance and the source of food borne resistant microbes, Referring to some examples of resistant bacteria in meat and milk products and the methods of detection of antimicrobial resistance, finally we would like to encourage some actions to Fight Antimicrobial Resistance by preventing the contaminations in food of animal origin by precise and continuous bacteriological examination. The aware use of antimicrobials, on all possible occasions, is the best way to mitigate the rise and dissemination of AMR among bacteria, particularly for drugs that are used in the remedy of both humans and animals. With greater alarming of the risks of indistinctive use of these antimicrobials, future issues in the treatment of pathogens can be controlled.

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