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## Assessment of bacterial diversity in the chicken litter: A potent risk to environmental health

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### ABSTRACT

Using chicken litter as an organic fertilizer on land is the most common, cheapest and environmentally safest way to manage the litter generated swiftly from the poultry industry. Raw chicken litter has been applied to field soils where various vegetables are cropped to increase yield or productivity. However, the chicken litter frequently come in contact with different environments, such as water, soil, microbes and vegetation. When chickens defecate, their litters, in a few countries, are particularly reused for the next flock, potentially causing cross-contamination. Due to various contact points in the environment, a high probability of bacterial transmission is predicted, which could lead to infection spread in animals and humans. Consumption of contaminated water, food, and meat could lead to the transmission of deadly infections. Microbes in the chicken litter also affect the grazing animals while feeding on fields duly applied with chicken litter as manure. The maximum permissible limits (MPLs) in the chicken litter for land application should not exceed  $10^6$ - $10^8$  CFU/g for Coliform bacteria. Antibiotics are regularly mixed in the diet or drinking water of chicken grown in marketable poultry farms for treating bacterial diseases. Rampant usage of antimicrobials also results in resistant bacteria's survival in animal excreta. Herein, we surveyed the literature to identify the major bacterial genus harboured in the fields applied with chicken manure to increase soil fertility. Our detailed survey identified different bacterial pathogens from chicken litter samples from different investigations. Most studies showed the prevalence of *Campylobacter*, *Salmonella*, *Enterococcus*, *E. coli*, *Bacillus*, *Comamonas*, *Proteus* and *Citrobacter*,

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including many other bacterial species in the chicken litter samples. This article suggested that chicken litter does not meet the standard parameters for direct application as organic fertilizer in the fields. Before being applied to the ground, chicken litter should be treated to lessen the danger of polluting crops or water supplies by reducing the prevalence of harmful bacteria carrying antibiotic-resistance genes.

## 1 Introduction

Poultry litter may have various environmental health issues when applied to agricultural land. Despite its role in improving soil fertility, it serves as a pipeline for transmitting bacterial species to the environment carrying different antibiotic resistance genes (ARGs) (Gurmesa et al. 2020; Kubasova et al. 2022). Antibiotics are used in chicken farming for treating infections caused by various microbes, including bacteria, fungi and protozoans and as growth promoters (Amarsy et al. 2021; Muhammad et al. 2022). Bacterial pathogens also carry ARGs and multidrug-resistant (MDR) genes in the digestive tract (food pipe to the gut) of farm animals and afterwards in their waste (IFT 2006; Subirats et al. 2020). Livestock manure/litter is the major origin of ARGs in the soil ecosystem in agricultural fields (Peng et al. 2017). Chander et al. (2007) stated that antibiotic resistance is one of the leading human health challenges, and it seems that the world is worryingly close to going back to the time before antibiotics. Due to the growing demand for meat products and eggs, poultry is one of the top-growing agro-based industries worldwide (Bolan et al. 2010; Kim et al. 2022).

Chicken excreta or litter is the waste created in huge amounts all over the globe (Aires 2009; Dornelas et al. 2023). Poultry litter remains a serious concern, and it is comprised of excrement, feathers, stray feed, dietary products and support matrix, which can differ as per the national and local conditions, like sawdust, shavings, nut shell, rice husk and others (Sanchuki et al. 2011). The chicken litter contains different pathogenic or non-pathogenic microorganisms, including bacteria, fungi, pathogenic protozoa, helminthes, and viruses (Figure 1). Besides carrying pathogenic microorganisms harboring antimicrobials resistance genes, chicken litter also possesses growth hormones, sex hormones like estrogen, testosterone and polycyclic aromatic hydrocarbons, heavy metals and pesticides (Deng et al. 2020). Traditionally, Antibiotics are used in broiler feed on typical or conventional farms to assist in rejuvenating growth and upgrade feed efficiency (Threlfall et al. 2000). Castanon noted that, as a result of recent developments in the study of MDR microbes, the U.S. government has stopped using antibiotics as a preventive measure, whilst the E.U. has banned their usage in poultry feed and as the growth promoters (Castanon 2007). Recently, microbial energy generation systems, i.e., bioelectrochemical systems (BESs), have been used as

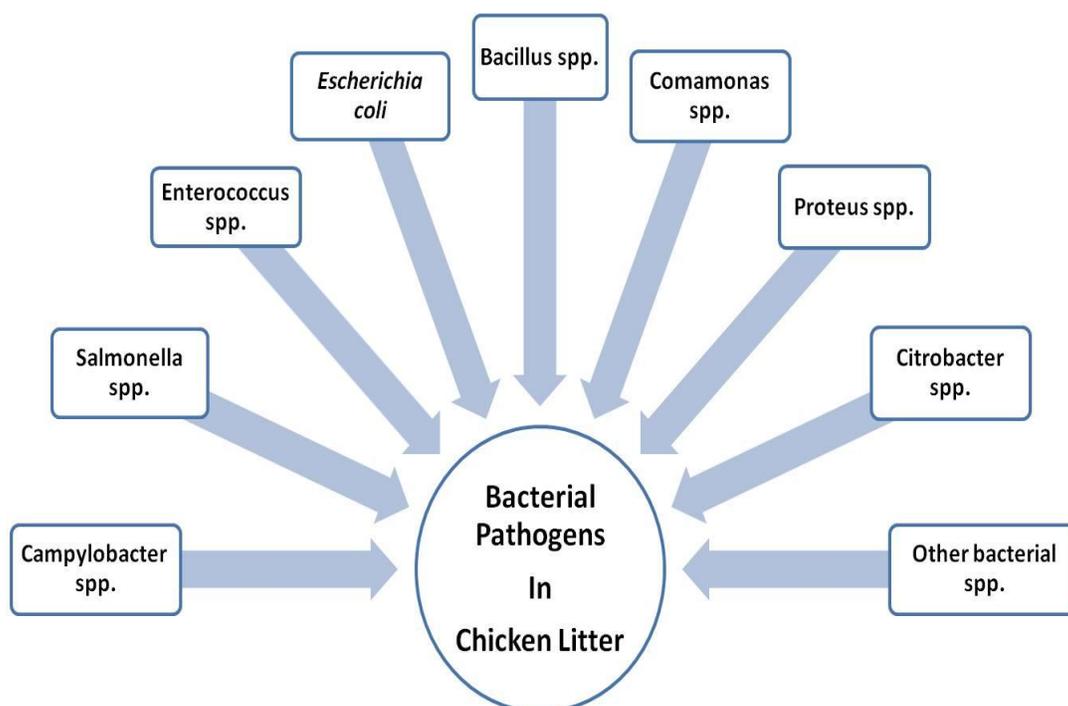


Figure 1 Different types of pathogenic or non-pathogenic microorganisms in the chicken litter

emerging sustainable technology with wide applications like wastewater treatment, heavy metal removal and biofuel production (Apollon et al. 2022). Such BESs systems can be proven helpful for managing chicken litter samples.

Antibiotic-resistant microbes spread into human and animal populations through a variety of routes like irrigation water, natural water, portable water and various eatables such as fruits, vegetables and other items (Kumar et al. 2021a; Kumar et al. 2021b; Kumar et al. 2021c; Kumar et al. 2021d; Kumar et al. 2022). Such resistant bacteria are passed in faeces, which can invade other organisms and share extrachromosomal genetic material called plasmids with neighbouring bacteria (Castanon 2007; Dhanarani et al. 2009; Olofinsae et al. 2022). Nsofor et al. (2021) gave systematic data that antibiotic use approaches are hardly followed in Nigeria, and antimicrobials are broadly utilized in the poultry industry without any check. Another study demonstrated that over 83% of poultry ranchers use tetracyclines, 50% use tylosin, 40% use gentamicin and under 30% use enrofloxacin, furazolidone, chloramphenicol, ciprofloxacin, penicillin, ampicillin and streptomycin (Dhanarani et al. 2009). Okonko et al. (2010) demonstrated that tetracycline is generally utilized in African poultry feeds, amongst other accessible antimicrobials. A recent study showed that poultry are of specific interest given their revealed linkage to vancomycin-resistant Enterococci diseases in humans (Fatoba et al. 2022). Antibiotic ratio is a significant element affecting ARGs (Bucher et al. 2020). After applying excrement or chicken litter as manure to fields, bacteria transmit from the animal's digestive tract to the environment carrying ARGs, influencing the environment and health of grazing animals in such areas (Subirats et al. 2020). To address the growing demand without a veterinarian's prescription, the U.S. Food and Drug Administration approved the use of antimicrobial agents as livestock feed supplements in 1951 (Rothrock et al. 2016). The present study aimed to survey the detailed literature for identifying the major bacterial genera that harboured in the chicken litter before being applied to the fields without treatment to increase soil fertility.

## 2 Bacterial Pathogens Found in Poultry Waste

Bolan et al. (2010) reported that microbial population density in poultry excreta or waste could outstrip  $10^{10}$  cells per g of litter, and gram-positive bacteria composed almost 90% of the microbial diversity. According to the available research, *Salmonella spp* and *Campylobacter spp* are those poultry bacteria that mainly cause human foodborne illnesses (Hafez 2005).

### 2.1 *Campylobacter spp.*

*Campylobacter spp* plays a vital role in foodborne diseases and is the leading cause of zoonotic enteric infections across the globe (Li

et al. 2022). Diseases caused by *Campylobacter* in humans are primarily transmitted through the food chain (Giaouris 2023). In the case of a known poultry house infection, there is no confirmation of horizontal or vertical transfer from one flock to another. Microorganisms, on the other hand, can be found in the guts of dead birds. As a result, environmental horizontal transmission appears to be the primary mode of *Campylobacter* infection in chickens (Dawson et al. 2023). Various slaughter techniques, such as shipping, de-feathering, and evisceration, increase the external *Campylobacter* load per bird (Hafez et al. 2014). Recently, the role of chicken litter in the ecology of *Campylobacter* has been revealed by Valeris-Chacin et al. (2022). They examined the association between litter microbiome and *Campylobacter* throughout the broiler production cycle. Another study suggested that management practices and environmental factors affected *Campylobacter* in the litter, which was linked with the number of flocks grown over broiler houses, litter, and pH (Oladeinde et al. 2023). The rampant rise of antibiotic-resistant *Campylobacter* demands advancing antibiotic-alternative methods to curb infections in humans and poultry. Recently, the ability of *E. coli* Nissle 1917 (EcN) was assessed to reduce *C. jejuni* colonization in chickens (Helmy et al. 2022). The effect of EcN on the intestinal morphology, immune responses, and gut microbes of chickens was measured. Another study was performed to address the existence of *Campylobacter* in chicken litter, which was reused for consecutive flocks (Raubert Wurfel et al. 2019). *C. jejuni* strain was traced as the leading one in the sentinel broilers and in the other environmental samples, suggesting a common and constant source of contamination within the flocks.

### 2.2 *Salmonella spp.*

*Salmonella* is found in a diversified range of foods but is most commonly found in animal products, particularly pig and poultry (Hugas and Beloeil 2014; Wojcicki et al. 2022). *S. typhimurium* is spread by infected meat, eggs, and dung, among other things (Asefa Kebede and Duga 2022; Igbinosa et al. 2023). When applied to croplands via animal manure, this bacterium, particularly those with antibiotic resistance, poses a major environmental and human health hazard that should be monitored closely (Jeamsripong et al. 2023; Malik et al. 2021). Australia's study showed contamination of 100% of broiler litter samples with *Salmonella* when reused as Organic Fertilizer on land (Kyakuwaire et al. 2019). Chicken litter is also found contaminated with a vast array of heavy metals and antibiotics in this study. Competitive exclusion factors are temperature and pH, which are crucial in influencing the level of pathogens in chicken litter. *Salmonella enterica* serovar Heidelberg (S.H.) has shown a high bacterial fitness over others, which survived for 14 days in the reused litter. The microcosm, incubation duration, and microcosm plus Heidelberg strain combination significantly affected the  $\beta$  diversity of the litter microbiome, including *Salmonella* (Bucher et

al. 2020). Little is known about how the horizontal gene transfer is affected by commensal microbes for antimicrobial resistance. Recently, a study showed that commensal bacteria contribute to reducing the horizontal gene transfer of antibiotic resistance to *Salmonella* (Oladeinde et al. 2022). Authors in this study used shotgun metagenomics and 16S rRNA gene sequencing to demonstrate that chicks were not at a high risk of being colonized by *Salmonella* Heidelberg strains when grown on reused litter.

### 2.3 *Enterococcus* spp.

Multi-drug-resistant bacteria such as vancomycin-resistant enterococci (VRE) have enhanced nosocomial infections in humans (Rafey et al. 2022; Simonetti et al. 2018). *Enterococci* are Gram-positive bacteria that are natural habitats of humans and animals' gastrointestinal tracts with a broad range of species such as *E. casseliflavus*, *E. faecalis*, *E. faecium*, *E. gallinarum*, *E. durans*, *E. mundtii*, *E. avium* and *E. hirae* (Zhou et al. 2020). Due to their frequent occurrence in human and animal faeces and prolonged environmental survival, *Enterococcus* spp. has developed into a widespread indicator of faecal contamination in the environment (WHO 2018; Dzelalija et al. 2023). Despite being regarded as commensal in humans, several *Enterococcus* species have been found as high-ranking (second only to staphylococci) agents responsible for nosocomial infections in humans (Haslam and St. Geme 2018). The presence of Enterococci is the marker of faecal contamination. A comparative examination of genome sequences revealed that *E. faecium* was found in fertilized soil, which is up to seven weeks older after manure application, as well as in exhaled dust (Rajendiran et al. 2022). Previously, comparable enterococci retention in manure-fertilized soil has been described. Genome sequencing of bacterial species had not been used before to track down the source of faecal contamination (Hodgson et al. 2016). A study from South Africa showed novel enterococci strains of antibiotic-resistant *Enterococcus* spp. by genomic analysis, which witnessed the transmission of plasmid-borne three AMR genes from chicken litter to agricultural land in South Africa (Fatoba et al. 2022). As per Pubmed search only seven studies has reported the incidence of *Enterococcus* spp. in the last five years, which suggested the need of more investigation for this bacteria in the chicken litter.

### 2.4 *Escherichia coli*

*E. coli* is a Gram-negative rod-shaped, not spore-forming bacteria. It is found in chicken litter samples harbouring multiple antimicrobial resistance genes (Khong et al. 2022). It could be mobile, i.e. through flagella or some other means, while others may be non-motile or non-flagellated. The bacterial species is a facultative anaerobe that can ferment simple carbohydrates like glucose to end products like produce lactic, acetic, and formic acids; the favourable pH for development is 6.0 to 8.0, but growth

can be observed at pH 4.3 and as high as pH 9 to 10 (Mitscherlich and Marth 1984). *E. coli* is an extensive and diversified bacterial group. Most *E. coli* strains are innocuous, but some strains have developed features, such as the ability to produce toxins, that render them hazardous to humans (Garcia et al. 2010; Woyda et al. 2023).

*E. coli* may survive in the environment for long periods and multiply in vegetables and other foods. According to the pathogenic mechanism, pathogenic *E. coli* has been divided into six types: (i) Enteropathogenic *E. coli* (EPEC), (ii) Enterohemorrhagic *E. coli* (EHEC), (iii) Enterotoxigenic *E. coli* (ETEC), (iv) Enteroaggregative *E. coli* (EAaggEC), (v) Enteroinvasive *E. coli* (EIEC) and (vi) Attaching and Effacing *E. coli* (A/EPEC) (Croxen et al. 2013; WHO 2015).

When chicken litter was used as manure, *E. coli* that were resilient to carbapenems and extended range beta-lactams might have gotten into the ecosystem, and the improper utilization of antibiotics as growth boosters in chicken and poultry diet may be the cause of this (Sebastian et al. 2021). A recent study investigated the antimicrobial resistance profile of *E. coli* isolates of chicken litter samples from pens in a broiler chicken experiment (Khong et al. 2022). This study showed high resistance to tetracycline, cephalothin, streptomycin, ampicillin, sulphonamides, colistin, and imipenem antimicrobials and 22% of *E. coli* isolates were observed with multidrug resistance (MDR) to at least three antimicrobials.

### 2.5 *Bacillus* spp.

*Bacillus subtilis*, *B. pumilus*, and *B. megaterium* are the bacteria generally found in fresh chicken litter but can also be a part of reused or old litter. Typically, these are used as prebiotic-based cleaning products. The results of this study show that treatment with P.B. can accelerate the naturally occurring process of diminishing populations of Enterobacteriaceae, which mainly contain the genus *Escherichia*, hence improving animal health and preventing poultry diseases (De Cesare et al. 2019).

### 2.6 *Comamonas* spp.

Four significant species of the genus *Comamonas*, *C. aquatica*, *C. kerstersii*, *C. terrigena*, and *C. testosteroni*, are found in poultry litter. These are the areas where the low-virulence diseases that occasionally affect humans and animals are caused by the cascade cage layers houses' nipple drinking mechanism (Chen et al. 2021).

### 2.7 *Proteus* spp.

*Proteus* species comes under the family Enterobacteriaceae of Gram-negative bacilli. According to reports, the bacterium *Proteus mirabilis* is one of the major causes of human pneumonia (Lysy et

al. 1985) and other infection conditions related to lungs (Wu et al. 2006). Further, 90% of *Proteus* infections are caused by *P. mirabilis*, typically in individuals with weakened immune systems (Cordoba et al. 2005). This infection can potentially progress endotoxin-induced sepsis, which causes systematic inflammatory response syndrome and has a 50% fatality risk. Additionally, it has been demonstrated that *P. mirabilis* can infect the CNS (Central Nervous System) (Kassim et al. 2003). The urea hydrolysis mediated by the enzyme urease produced by this bacterium leads to the development of urolithiasis. The hydrolysis causes an increase in pH that results in the precipitation of crystals of Calcium Phosphate (apatite) and magnesium ammonium phosphate (struvite), which obstructs the urinary system (Mobley and Warren 1987). *P. mirabilis* also contributes to the inability of some avian species to reproduce (Cabassi et al. 2004). Additionally, *P. mirabilis* has been linked to animal kidney and urinary tract infections in earlier studies (Greenberg et al. 2004).

Research on the biochemical responses of human *P. mirabilis* isolates, and the degree of variation in their traits has been extensively published in the literature (Bergey and Holt 1993). Unfortunately, very scant information is available about *P. mirabilis*, which is of animal origin. Finding biochemical similarities between organisms causing illnesses in humans and animals is crucial, mainly when the biochemical trait is also a virulence factor, like the urease enzyme in *P. mirabilis*, which contributes to the development of kidney stones (Li et al. 2002).

### 2.8 *Citrobacter* spp.

*Citrobacter* genus bacteria and *Salmonella* share similarities in their cell surface antigens and biochemical characteristics because of their close kinship. Pillar et al. (2020) confirmed the close association of *Citrobacter* and *Salmonella* by demonstrating that they share 33% common core genes at the genome level. Their shared evolutionary history and genetic exchange can account for this unusually high genotypic resemblance. *Citrobacter* spp could be mistaken for *Salmonella* due to the characteristics mentioned above. It is also crucial to note that it takes an additional day or two to confirm ambiguous identifications, delaying the results and driving up the cost of analysis (Retchless and Lawrence 2010). *Citrobacter* spp was the second most prevalent bacteria to be retrieved and recognized from chicken litter during research, following *E. coli*, out of 149 isolates (Meshref et al. 2021).

### 2.9 Other bacterial species

Culture-based bacterial identification indicated *Enterococcus* spp. and *Coliforms*, but culture-independent approaches revealed additional bacteria such as *Globicatella sulfidofaciens*, *Corynebacterium ammoniagenes*, *C. urealyticum*, *Clostridium aminovalericum*, *Arthrobacter* spp and *Denitrobacter*

*permanens*. When chicken litter DNA samples were utilized as templates for microbial diversity evaluation, other harmful bacterial species such as *Clostridia*, *Staphylococci*, and *Bordetella* spp were also discovered (Meshref et al. 2021). A study from the USA reported the microbial diversity associated with offensive odour modification for the production of fertilizers from chicken litter in the USA (Enticknap et al. 2006). Bacterial strains such as *Atopostipesuicloacalis*, *Aerococcus viridians*, *C. ammoniagenes*, *Facklamia sourekii*, *Brevibacterium avium*, *Jeotgalicoccus* spp, *Salinicoccus halodurans*, *Virgibacillus marismortui*, *Staphylococcus arlettae*, *Staphylococcus cohnii* and *Bacilli hackensackii* were found to be prominent in both wet and dry poultry litter samples, out of which *Staphylococcus*, *Salinicoccus*, *Virgibacillus*, *Jeotgalicoccus*, *Facklamia*, *Brevibacterium* and *Bacilli* were found to be dominant (Wadud et al. 2012).

The impact of fresh or used excrement on the maturation of broiler chicken's immune systems has recently been described, in addition to the relationship between the kind of litter and gut microbiota, demonstrating the intricate relationship between the development of immune cells and the type of litter used for chicken growth (Lee et al. 2011; Torok et al. 2009).

### 3 Conclusions and Future Prospects

This study showed that multiple bacterial species have been identified from poultry litter because the gut of animals acts like their habitat. Various bacterial species have previously been isolated from chicken litter in many investigations where *Enterococcus faecium*, *Proteus mirabilis*, and *Campylobacter jejuni* have been identified as the most common ones. These pathogenic bacteria transmitted to the environment cause several diseases in animals and humans after coming in contact with infected chicken litter. Studies have also proved that some are responsible for transmitting antimicrobial resistance genes in the environment when they come in contact through any means. There is a dire need to periodically perform environmental safety surveys to trace harmful bacteria in the region where chicken litter is regularly applied in the agricultural fields.

This study highlighted the presence of different bacteria in the chicken litter in the other geographical regions. An economically viable solution is highly suggested for waste disposal from chicken houses to produce a natural fertilizer by a suitable treatment procedure, which can be commercially marketed for garden and commercial use. Treatment or modification of chicken litter includes enzymatic treatment and addition of *Streptomyces* sp. spores during composting, which may lead to achieving maximum benefits. The future directions underscore the efforts towards effectively converting chicken litter into a valuable fertilizer to alleviate the negative environmental effects.

**Conflict of interest disclosure**

Authors declare no conflict of interest.

**Availability of data and material**

Data is transparent and will be available when required.

**Code availability**

Custom codes.

**Author contributions**

Sunil Kumar planned the study. Mukesh Yadav, Nirmala Sehrawat & Tamanna Devi drafted the manuscript. Razique Anwer monitored the content and arranged the references. Razique Anwer, Anil Kumar Sharma & Neera Mehra reviewed the grammar and English check manuscript.

**Ethics approval**

Not required.

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