

ZOONOTIC BACTERIA IN THE VICINITY OF ANIMAL FARMS AS A FACTOR DISTURBING THE HUMAN MICROBIOME: A REVIEW

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Abstract

This review is aimed at summarizing the current state of knowledge about the relationship between environmental exposure to the bioaerosol emitted by intensive livestock farming and changes in the microbiome of people living in livestock farm vicinity. The PubMed, Scopus and Web of Science databases were searched by crossing keywords from the following 3 groups: a) “livestock,” “animal farms,” “animal breeding”; b) “microbiome,” “resistome”; c) “livestock vicinity,” “farm vicinity,” “neighborhoods and health” in 2010–2022. Literature screening did not reveal any paper related to the full microbiome composition in the population studied. In the study, the authors included 7 papers (5 from the Netherlands, 1 from the USA, and 1 from China). The studies confirmed the carriage of *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA), livestock-associated MRSA (LA-MRSA MC398) and multidrug-resistant *S. aureus* (MDRSA) in the nasal microbiome of adults and children living within 500–2000 m from a livestock farm. *Clostridium difficile*, including LA-ribotype RT078 carriage, was detected in the intestinal microbiome of adults living within 500–1000 m. Extended-spectrum β -lactamase (ESBL) producing *Enterobacteriaceae* were confirmed in the intestinal microbiome of adults living within 500–6200 m. Knowledge on the composition of the microflora of people living in livestock farm vicinity is insufficient to conclude about changes in the microbiome caused by the environmental emission of bioaerosol. The carriage prevalence of the LA-bacteria, including both strains with antimicrobial resistance and antimicrobial resistance genes, confirms the presence of zoonotic bacteria in the human microflora in populations without occupational contact with animals. It cannot be ruled out that zoonotic bacteria, as a component of the microbiome, have a negative impact on people's health. Int J Occup Med Environ Health. 2024;37(2)

Key words:

environmental exposure, microbiome, livestock, antibiotic resistance microorganisms, ARG, farm vicinity

INTRODUCTION

The microbiome and the resistome, including in relation to humans, animals and their habitats, are currently in the focus of experts in the field of environmental health [1,2]. Large industrial animal farms are a particular microbial environment due to specific conditions related to large or extremely large herds, reaching up to even hun-

dreds of thousands of animals living in a limited area [1]. Animals gathered in such numerous herds in 1 location produce large amounts of manure strongly contaminated by zoonotic microorganisms, including antibiotic resistant bacteria (ARB) and multi-antibiotic resistant bacteria (MARB) [3].

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The common use of antimicrobials in livestock farming for many years has caused an extremely large dissemination of antimicrobial resistance in bacteria colonizing both the animals and farm environment [4,5]. This thesis has been proven in the studies on antimicrobial resistance in bacteria isolated from livestock, in which the domination of tetracycline resistance genes, a drug widely used in veterinary medicine, has been found [6]. Although currently the use of antibiotics in livestock farming is legally permitted only for therapeutic purposes, it is confirmed in several studies that antimicrobial resistance genes (ARGs) are also present in the microbiome of animals that have never been treated with antibiotics [6].

The bacteria impact does not end at the farm but goes far beyond and strongly influences the microbiome of the entire microenvironment located in the certain radius from the emitter [1,7]. There is strong evidence that zoonotic bacteria are present in the soil, water and air around livestock farms [1]. Based on the results of the epidemiological studies conducted both in Europe and in other continents, it is impossible to exclude that biological agents originating from farm animals have an harmful impact on the health of populations living in the farm vicinity [8,9]. Zoonotic bacteria can cause local infections limited to the skin or other organs as well as systemic infections. Children and the elderly are vulnerable populations, and the highest risk of infections concerns individuals with immunodeficiency caused by other disease processes [8]. Another problem in the farm vicinity are microorganisms carrying ARGs, which are transferred in high concentrations to the soil and groundwater with manure used as a fertiliser in crops, leading to further spreading of antibiotic resistance (AR) in the environment [10].

According to the latest knowledge, the human microbiome composition is a critical factor for the health status of an individual [11]. Human health is a result of compound, interconnected actions, a network of interac-

tions between microbes and their host [12]. Studies have shown that urban residents have a lower microbiome diversity compared to rural inhabitants [13,14]. People living in livestock farm vicinity can become vectors of ARB and MARB strains of the zoonotic pathogen and transmit them to the general population. One of the most significant effects on public health is their embedding in hospitals, where they are the cause of nosocomial infections [13]. Currently scientists notice an additional problem referring to changes in the human microbiome as a consequence of contact with the zoonotic bacteria and ARGs emitted by farms to the environment [14]. Dysbiosis in the composition and functions of the gut microflora is associated with chronic diseases, from the inflammation of the gastrointestinal tract and metabolic diseases to the neurological, cardiovascular and respiratory diseases [15]. Although reports confirming the occurrence of zoonotic bacteria, ARB and ARGs in the livestock environment, along with the microbial transmission from animals to farm workers, are more and more frequent, there is a knowledge gap in recognising the scale of changes in the microbiome of populations living in livestock farm vicinity, but without occupational contact with the animals. The presented narrative literature review is aimed to summarize the current state of knowledge about the relationship between environmental exposure to the bio-aerosol emitted by intensive livestock farming and changes in the microbiome of people living in livestock farm vicinity regardless of the breeding animal species.

METHODS

The microbiological burden in the farm environment depends on animal species and the type of livestock farms. For instance, poultry and pigs are hardly comparable. The text of the narrative review is focused on the possible changes in the human microbiome as the effect of environmental exposure to microorganisms originating from livestock, regardless of animal species, and not

on specific changes caused by specific microorganisms originating from particular animal species.

The literature search of the relevant papers was conducted in 2010–2022 using the PubMed, Web of Science and Scopus databases. This period was chosen to reflect findings over the past 10 years, in which the molecular methods of microbial identifications have come into common use, and the knowledge about the association between the microbiome composition and human health has significantly increased.

The search strategy was based on combined terms referring to the title and aim of the study, as follows:

- “livestock,” “animal farms,” “animal breeding”;
- “microbiome,” “resistome,” “carriage”;
- “livestock vicinity,” “farm vicinity,” “farm neighborhoods.”

The specific terms used in the review:

- “vicinity” means the direct area surrounding the livestock farm (with or without a determined diameter) or people living in that area, according to the cited article;
- “intensive livestock farming” is equal to “concentrated animal feeding operations” – animal feeding operations conducted on a large scale and aimed at the highest economic profit [9];
- “small scale production” means the husbandry aimed at putting one’s animals into the best possible environment to meet their physical and psychological needs, and then augmenting their ability to survive and thrive by providing them with food, protection from predation, water, medical attention, help in birthing, and so on [2].

The original and review papers identified by combining the above keywords and by reviewing the references cited therein were included in the narrative review. Articles referred to the microbiome of animal keepers (farm workers) and livestock animals were excluded. Another exclusion criterion was the date of publication before 2010.

Irrelevant studies were excluded. The remaining 7 articles were subject to a full-text review. The background and discussion sections were prepared based on the literature items that were included in the queries results but did not show the main criterion, i.e., a study on the microbiome of people living in livestock farm vicinity.

RESULTS

Literature screening did not reveal any paper related to the full microbiome composition in populations living in livestock farm vicinity. Papers selected to the review present the results of studies on the carriage of particular species/strains of zoonotic bacteria in the nasal or intestinal microbiome. The results below only relate to the microbiomes of people living in the vicinity of animal farms, even if these results were a part of larger studies conducted in the livestock environment. In Table 1, the authors presented the profile of data and results relating to the studies on the microbiome of people living in livestock farm vicinity, published in the papers meeting the review criteria.

Three studies included in the review concern the association between the zoonotic bacteria emission from livestock farms and the presence of *Staphylococcus aureus* species in the microbiome of community residents, particularly methicillin-resistant *S. aureus* (MRSA) strains. In the USA, a study was conducted in the area of a high density of industrial hog operations (IHOs) aimed to compare *S. aureus* carriage, including MRSA and multi-drug-resistant *S. aureus* (MDRSA), and livestock associated (LA) *S. aureus* (LA-*S. aureus*) including *scn*-negative *S. aureus* (strains without the gene coding staphylococcal complement inhibitor with anti-phagocytic activity considered as the livestock host-specific genetic marker), CC398 and CC9 clonal complex (CC), in the populations of farm workers living in IHOs vicinity (the study group) and community residents without occupational exposure to IHOs (the control group). It was shown that the

Table 1. Carriage of zoonotic bacteria in the microbiome of people living in livestock farm vicinity without occupational contact with animals, based on world literature dating 2010–2022

Study	Study population	Livestock	Farm–household distance [m]	Participants	Material	Method of microbial identification	Prevalence [%]
China [18]	adults living in the vicinity of a slaughterhouse	pigs	500, 1500, 2000	59 adults	nasal swabs	conventional culture on selective media; WGS; PCR; broth microdilution	MSSA – adults 11.86 – children n.a. MRSA – adults 6.78 – children n.a.
USA [16]	adults and children living in an area of a high density of farming, without occupational exposure to IHOs	pigs	not specified	202 adults, 202 children (202 households)	nasal swabs	conventional culture on selective media; PCR/MALDI-TOF MS	<i>S. aureus</i> – adults 31.00 – children 31.00 MRSA – adults 3.00 – children 6.00 MDRSA – adults 8.00 – children 8.00
The Netherlands [17]	adults living in the vicinity of an area of a high density of farming	pigs, poultry, cows, horses, sheep	500, 1000	2492 adults	nasal swabs	conventional culture on selective media; PCR	MRSA – adults 0.56 – children n.a. LA-MRSA (MC 398) – adults 71.43 ^a – children n.a.
The Netherlands [21]	adults living in the vicinity of an area of a high density of farming	pigs, poultry, cows, horses, sheep	500, 1000	2432 adults	faeces	conventional culture on selective media; PCR	<i>C. difficile</i> – adults 1.23 – children n.a. <i>C. difficile</i> RT 078 – adults 0.16 – children n.a.

The Netherlands [22]	adult patients hospitalized with CAP	poultry	<1000, ≥1000	126 (100 ≥1 km; 26 <1 km) ^b adults	oral cavity and throat swabs	high-throughput sequencing (16S-rRNA)	<i>S. pneumoniae</i> ^c – adults 34.90 (<1 km), 22.50 (≥1 km) – children n.a. <i>Lactobacillus</i> ^c – adults 1.40 (<1 km), 3.80 (≥1 km) – children n.a.
The Netherlands [19]	adults living in the vicinity of an area of a high density of farming	pigs, poultry, cows, horses, sheep	500, 1000	2432 adults	faeces	conventional culture on selective media, biochemical test, disc-diffusion test, PCR	<i>E. coli</i> – adults 4.19 – children n.a. <i>K. pneumoniae</i> – adults 0.21 – children n.a. ESBL/pAmpC-E – adults 4.50 – children n.a. ESBL-ARGs – <i>bla</i> _{CTX-M-15} ○ adults 42.20 ^d ○ children n.a. – <i>bla</i> _{CTX-M-14/17} ○ adults 17.43 ^d ○ children n.a. – <i>bla</i> _{CTX-M-1} ○ adults 11.93 ^d ○ children n.a. pAmpC genes – <i>bla</i> _{CMV-2} ○ adults 8.26 ^d ○ children n.a. – <i>bla</i> _{DHA-1} ○ adults 0.92 ^d ○ children n.a.

Table 1. Carriage of zoonotic bacteria in the microbiome of people living in livestock farm vicinity without occupational contact with animals, based on world literature dating 2013–2022 – cont.

Study	Study population	Livestock	Farm–household distance [m]	Participants	Material	Method of microbial identification	Prevalence [%]
The Netherlands [20]	adults living in an area of a high and low density of broiler farms	broilers	2200 (high density), 6200 (low density)	1025 adults (533 – high density/492 – low density)	rectal swabs	conventional culture on selective media, biochemical test, disc-diffusion test	ESBL-producing <i>Enterobacteriaceae</i> – adults 3.60 (high broiler densities) – 6.70 (low broiler densities) – children n.a.

n.a. – data not available.
 ARGs – antimicrobial resistance genes; CAP – community-acquired pneumonia; ESBL – extended-spectrum β-lactamase; IHOs – Industrial Hog Operations; LA-MRSA – livestock-associated MRSA; MALDI-TOF MS – matrix-assisted laser desorption ionization time-of-flight mass spectrometry; MDRSA – multidrug-resistant *S. aureus*; MRSA – methicillin-resistant *S. aureus*; MSSA – methicillin-susceptible *S. aureus*; PCR – polymerase chain reaction; pAmpC-E – plasmid-encoded AmpC β-lactamase resistance genes of Enterobacteriaceae (*E. coli*/*K. pneumoniae*); WGS – whole-genome sequencing.
^a The prevalence of LA-MRSA in the population of MRSA carriers. ^b Residential proximity to a poultry farm. ^c A relationship between a higher abundance of *S. pneumoniae* and a lower abundance of *Lactobacillus*.
^d The percentage values refer to the participants being carriers of ESBL/pAmpC-E (N = 109).

prevalence of *S. aureus*, MRSA and MDRSA in the nasal microbiome was on the same level in adults and children living in the shared household (detailed results in Table 1). The carriage of LA-*S. aureus* strains including *scn*-negative *S. aureus* (2% of all carriers) and *scn*-negative MDRSA (1%) was also found. There was no confirmed nasal carrier of *scn*-negative MRSA. The nasal carriage prevalence of *S. aureus* CC398 and CC9 was found in 1% of adults in both cases. All CC398 and CC9 carried by the participants were *scn*-negative. In children, the prevalence of nasal *scn*-negative *S. aureus*, *scn*-negative MRSA, and *scn*-negative MDRSA was low (2%, 0%, and 1%, respectively). The prevalence *S. aureus* CC398 (*scn*-negative) and *S. aureus* CC9 (*scn*-negative) in children reached 1% (2 children and 1 child, respectively). It was observed that the participants without occupational contact with livestock but living in areas of a high density of IHOs are sometimes carriers of the bacteria in the nasal microbiome [16].

A study conducted in the Netherlands investigated the nasal MRSA carriage among adults (18–70 years old) living in a highly populated rural area with a high density of livestock farms but not living or working on a farm. The study group involved 1 participant per household. The study analyzed the prevalence of participants' colonization by the MRSA and LA-MRSA strains. The study group was composed of 2492 persons among whom only 14 persons were identified as carriers of MRSA strains. Most of them (10 persons) were carriers of LA-MRSA strains and these individuals lived significantly closer to the nearest farm than non-carriers. It was confirmed that living significantly closer to the nearest livestock farm is a risk factor for LA-MRSA carriage, which is probably caused by environmental exposure [17].

A cross-sectional study carried out in China investigated both MRSA and methicillin-susceptible *S. aureus* (MSSA) transmission from pigs to pork in the environment and surrounding community, including community residents living in the vicinity of pork production chain points. Nasal

swabs were taken from 59 adult residents, about 20 people living in each of the 3 communities located: 0.5 km from the slaughterhouse, and 1.5 km and 2 km from pig farms. Both the MRSA and MSSA strains were detected in residents from the surrounding community (4/59 and 7/59, respectively). Although LA-MRSA CC9 strain previously reported in the pig farm environment in China was detected in the pork production chain (pig farms, the slaughterhouse and the pork wholesale market), it was not identified in the nasal swabs collected from community residents. The dominating strain in the isolates from community residents was MRSA ST59-t437, described previously in China as a community-associated MRSA (CA-MRSA) strain. The MRSA identified in humans in the surrounding community and villages belonged to ST59-t437, which is a common CA-MRSA strain in China. The MRSA isolates from humans were resistant to a smaller number of antimicrobial agents in comparison with the MRSA isolates from pigs, and the difference was statistically significant. In the case of MSSA isolates, there was no specific phenotype of resistance linked to their origin. The results indicate that, in the study area, at least few separated MRSA isolate populations are present, linked with pigs (mainly CC9), humans with occupational contact with pigs (mainly ST59), and humans living in pork production chain vicinity (mainly ST59-t437) [18].

Two papers included in the review concern the carriage of *Enterobacteriaceae* in the human gut based on rectal swabs or faeces samples.

A study conducted in the Netherlands tested the prevalence of the gut carriage of extended-spectrum β -lactamase (ESBL) and/or plasmid-mediated AmpC-producing *Enterobacteriaceae* (pAmpC-E) in the general population living in a livestock-dense area. Associations between this carriage and exposure through the animal contact and the environment were investigated. The study population consisted of 2432 participants, providing faeces samples. The carriage of ESBL/pAmpC-E was determined

in 109 out of 2432 residents, ranging 1.4–10.9% in the locations of 12 geographically different research centers. The ESBL/pAmpC resistance genes were detected in isolates of *Escherichia coli* (identified in 102 participants) and *Klebsiella pneumoniae* (5 participants); both these species were detected in 2 participants. The most common ESBL resistance genes were *bla*_{CTX-M-15}, *bla*_{CTX-M-14/17} and *bla*_{CTX-M-1}, determined in the samples obtained from 76 participants; and the most common pAmpC genes were *bla*_{CMY-2} and *bla*_{DHA-1} identified in 9 and 1 participants, respectively. Living within 1000 m from a mink farm was a significant risk factor of carrying resistance genes. What is important, exposure to poultry was not identified as a risk factor. The environmental exposure to livestock was not the cause of a higher prevalence of ESBL/pAmpC-E carriage in the population living in livestock farm vicinity [19].

A study also conducted in the Netherlands determined the prevalence of, and risk factors for, the carriage of ESBL-producing *Enterobacteriaceae* in populations living in areas of either high or low broiler densities. People living or working on commercial broiler farms were excluded from the study. The study was conducted based on rectal swabs from 1025 adults living in selected municipalities with either high (533 individuals) or low (492 individuals) broiler densities. The results have shown statistically significant differences in the prevalence of ESBL carriage, contrary to what was expected, while carriage in areas of low broiler densities was higher (33 ESBL-positive individuals out of 492 in total) than in areas of high broiler densities (19 out of 533, respectively). What is more, in populations living in areas of high broiler densities, the average distance to the nearest broiler farm was also significantly smaller than in the population living in an area of low broiler densities (2.2 km, SD = 1.4, range 0.2–7.3 vs. 6.2 km, SD = 2.9, range 0.9–11.8). The multivariable statistical analysis did not indicate the possibly causative factors of an extended risk of ESBL carriage for people living in areas of both high and low broilers farm densities. However, the contact with

a horse or other companion animals significantly increased that risk. According to the authors, the overall prevalence of 5.1% (52 positive individuals out of 1025 in total) was comparable with other countries, which indicates the prevalence in the general community in the Netherlands without a link to the environmental exposure to strains with antimicrobial resistance (AMR) or ARGs from broiler farming [20].

One paper concerns the gut carriage of *Clostridium difficile*. A large study conducted in the Netherlands investigated the prevalence of *Clostridium difficile* in human faeces samples delivered by residents of an area of a high density of livestock farms. It was found that, among the study participants, only 30 individuals were carriers of *C. difficile*. The study analyzed the prevalence of toxin-producing ribotypes, in particular ribotype 078 which is the most prevalent in livestock [21]. The identified strains belonged to different ribotypes, including:

- toxin-producing ribotypes (N = 21): ribotype 078 (N = 4), ribotype 014 (N = 7), ribotype 002 and ribotype 265 (N = 2 each), ribotype 001, ribotype 005, ribotype 012, ribotype 013, ribotype 017, and ribotype 024 (N = 1 each),
- ribotypes not producing toxins (N = 9): ribotype 039 (N = 4), ribotype 010, ribotype 085, ribotype 071 (N = 1 each), and an unknown ribotype (N = 2).

In the statistical analysis, factors based on the livestock environmental exposure were included and *C. difficile* carriage could be linked (e.g., gastroenteritis in the month preceding the study, the frequency of nausea, diarrhea and fever, antibiotics use in 3 months before the study, hospitalization in the past 12 months; travelling to high *C. difficile* risk countries, contact with farm animals). It was shown that *C. difficile* colonization was associated with gastroenteritis in the month preceding the study and with antibiotics use in 3 and 6 months before the study while there was no relationship between *C. difficile* carriage and living in an area of a high density of livestock farms [21].

The last paper included in the review shows a different approach to the investigated topic than the above-discussed research. The study was conducted based on the population of community-acquired pneumonia (CAP) patients living in livestock farm vicinity, instead of healthy individuals. The study was carried out in the Netherlands and investigated the increased risk of CAP near poultry farms based on the analysis of the oropharyngeal microbiota composition in the studied patients, related to the residential proximity to poultry farms. The hypothesis was that CAP and living near poultry farms are associated through a direct effect of environmental emissions from livestock on the microbiota composition within the human respiratory tract, including colonization by zoonotic pathogens. The study compared the microflora compositions between the populations of CAP patients living in the proximities of <1 km and ≥ 1 km from the nearest poultry farm. It was found that the statistical association between living within the proximity of 1 km from a poultry farm and the overall oropharyngeal microbiota composition is on the borderline significance. A study of the oral and oropharyngeal microbiome in CAP patients found an association between increased *S. pneumoniae* and decreased *Lactobacillus* relative abundance in people living <1 km from the farm, compared to those living further away. In the case of other bacterial species identified in the respiratory microbiomes, no statistically significant associations with residential proximity to poultry farms were revealed. Nonetheless, the excess risk of CAP pneumonia (about 11% increase) was demonstrated in areas of approx. 1.15 km around poultry farms. The major limitations of the study were including only CAP patients, without a healthy control group, and a small size of the population studied. The results indicate that there is a need to conduct a large-scale study on the relationship between the respiratory microbiome and the prevalence of CAP in people living in livestock farm vicinity [22].

DISCUSSION

The papers included in the review describe the carriage of pathogens, opportunistic strains and/or ARGs but not the full spectrum of bacterial species/strains present in the human microbiome. It should be noted that only 7 papers were found that matched the criteria and responded to the aim of the study. The research field in the presented topic is limited due to the fact that livestock farms are usually located in rural areas, where the population of farmers predominate, which is associated with a high probability of infections contracted from their own animals or by occupational contact with livestock [21]. The Netherlands is one of the world's most densely populated countries with a remarkably high concentration of intensive livestock farms. The common localisation of industrial livestock farms in densely populated areas is described by researchers from the Netherlands as a "natural experiment" [23]. Therefore, 5 out of 7 papers included in the review were carried out in the Netherlands. The antimicrobial resistance as the potential human health threat is listed among the most significant challenges for the public health around the world [23]. In some papers [16], the prevalence of ARB/ARGs carriage in humans living in livestock farm vicinity was included in the study as the control group for the population of livestock workers. Nevertheless, all studies presented in the review should be recognised as an important part of the microbiome investigation of people living in livestock farm vicinity. The human microbiota is the community of commensal and symbiotic microorganisms occurring on the skin, in the gastrointestinal tract, the respiratory system and the urogenital system, but also opportunistic microorganisms that, under some conditions, can cause host infections [11]. The human microbiome is shaped by both internal and external factors, and it evolves and changes its composition over a lifetime by adapting to changes in environmental factors (such as diet, stress level, microorganisms present in the human

living environment, taken drugs, etc.) [11]. The more opportunistic and pathogenic zoonotic bacteria present in the human microflora, the higher the risk of infection for the host [11]. All bacteria isolated from humans living in livestock farm vicinity, including *S. aureus*, *C. difficile*, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, and other strains of *Enterobacteriaceae*, have a high pro-inflammatory potential, which increases the probability of microbiome dysbiosis [11,23]. It is currently known that the disturbance of the normal microflora interaction increases the host's susceptibility to disease [11,24]. Dysbiosis of the gut microbiome may lead to the development of many diseases and health issues, include autoimmune disease, brain dysfunction, learning difficulties, mood disorders (depression, aggression, bipolar affective disorder, anxieties, psychosis and schizophrenia), joint pain, allergic and inflammatory reactions, food allergies, asthma, respiratory infections, heart diseases, and cancer development [11]. The following genera are among the zoonotic pathogens identified in the livestock environment: *Staphylococcus*, *Salmonella*, *Campylobacter*, *Enterococcus* and strains of *E. coli* species. These pathogens are transmitted to the farm vicinity via the air (organic dust) and surface water (liquid manure, animal faeces). Some of them are able to survive for a long time in an active form, being a potential human infectious agent and a source of antibiotic resistance genes [6]. Organic dust, being a carrier of bacterial bioaerosol, is transmitted by the wind over long distances [7,25]. Studies on bioaerosol emission from farm buildings to large distances confirm the exposure of residents to such harmful agents as zoonotic bacteria and antibiotic resistant genes. In the Netherlands, in an area of a high density of farms, it was shown that both bacterial DNA originating from zoonotic commensals and antibiotic resistance genes were detectable in residential sites located 1200 m from farms [26]. People living in the farm vicinity are exposed to much lower concentrations of organic dust than farm workers, but it should be

expected that these microorganisms will colonise their microbiomes [1]. However, AMR is the biggest challenge. The scale of the problem can be estimated by deaths worldwide, which suggest that at least 700 000 deaths/year are caused by infections induced by drug resistant strains of common bacteria species. In fact, experts agree that these estimates are rather cautious and the numbers are heavily underestimated [6,27]. Multidrug resistance in the past 2 decades has reached the pandemic level [28]. Thus, the constantly growing trend for the number of ARB and MARB strains rises concerns [5–7]. It should be noted that AMR is linked to many causative agents but the most significant one is the excessive use of antibiotics both in medicine and veterinary medicine [6]. The acquired resistance is developed through various mechanisms as a result of:

- the selection of resistant strains by eliminating sensitive strains,
- genetic mutations,
- the transfer of genetic information from drug-resistant bacteria,
- cross-resistance resulting from incorrectly dosed antibiotics [6].

Bacteria are also capable of acquiring AR through horizontal gene transfer that occurs through mobile genetic elements such as plasmids and transposons [6,29]. Plasmid transfer takes place between phylogenetically distant bacterial species in both groups, the environmental bacteria and pathogens [30]. This means that ARGs, being a result of the inappropriate use of antibiotics in animal farming, can easily and rapidly spread to all other bacteria species, including other strains in the human microflora [3,29].

In the field of public health, the most important pathogens are grouped under the acronym ESKAPE that is composed of the first letters of the 6 bacterial species and generic names (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,

Pseudomonas aeruginosa, and *Enterobacter* spp.) [31]. Both ARB and ARGs are transmitted between humans, animals and the environment; if they are present in one of these settings, they will be a threat to the rest of them, which is called the One Health approach [31]. Species that are both human and livestock opportunistic pathogens, such as *S. aureus*, *E. coli* and *C. difficile* [17,21,22], can be used as reliable indicators of the impact of environmental exposure on the microbiome of people living in farm vicinity.

The majority of presented studies focus on adult people living in livestock farm vicinity, and only 1 paper includes both adults and children living in the same households. As other researchers have noted [16], it is important to recognise the carriage of zoonotic ARB, particularly *Staphylococcus aureus*, in the population of children living in industrial livestock vicinity or in families whose adult members have occupational contact with farm animals. Young children are more susceptible to *S. aureus* infections than other family members due to the nature of contact with parents and other adult relatives (hugs, close conversations, kisses) and the stage of life in which their immune system is intensively developing [16].

Staphylococcus aureus is the natural component of both human and livestock microflora but this species is also the opportunistic pathogen with much significance to public health. This species is particularly susceptible to the development and acquisition of antibiotic resistance. The MRSA and MDRSA, including LA-MRSA strains, are indicated as very important causative agents of nosocomial infections [16–18]. The review indicates that the prevalence of MRSA is higher in China and the USA than in the Netherlands [16–18] but the important limitation is the low number of studies. The LA-MRSA strains exhibit a high degree of survival in adverse environments and are able to survive in organic dust for even 2 months. The *Enterobacteriaceae* strains producing ESBL are also among the alarming pathogens that pose a health hazard

for residents of areas in farm vicinity [10,31]. The hypothesis that intensive livestock farming is a source of clinically significant ESBL bacteria is confirmed by a series of molecular studies conducted on bacterial strains isolated from humans, livestock and food [31,32]. Many genetic similarities between ESBL strains isolated from humans and poultry were detected in the Netherlands [32]. The occurrence of the same genetic lineage of ESBL in both manure and airborne isolates was confirmed in the broiler farm environment in Spain [33].

The study aimed at investigating the ESBL/AmpC-producing *E. coli* transmission from broiler farms to the environment was carried out in Germany. A comparison of bacterial isolates from inside the broiler environment (samples of faeces and air inside animal houses) with isolates from outside broiler farms (a boot swab 50 m downwind and an air sample 50 m downwind) showed 100% genetic similarity [34]. The same authors confirmed the possibility of the ESBL/AmpC-producing *E. coli* strains transmission from pig farms to the surrounding environment via slurry, air and flies. Animal waste is the natural environment for house flies, which lay their eggs in faeces, resulting in ARB and ARGs transmission. As house flies can move many kilometres each day, those living in the livestock farm environment should be treated as a human health threat [35]. It was confirmed in the Netherlands that people living in livestock farm vicinity are the carriers of ESBL strains, but in the case of broilers breeding, the prevalence of ESBL-producing *Enterobacteriaceae* strains was higher in the area of a low density of poultry farms [19,20]. This association is rather surprising and difficult to explain based on current knowledge.

Another bacterial species associated with livestock farming is *Clostridium difficile*. Over the previous decade many new strains of *C. difficile* were isolated from various environments but 1 of them, i.e., ribotype 078, has been identified in large numbers in piglets, calves and their surrounding environment. This isolate was marked

as LA and becomes an indicator of zoonotic bacteria pollution in the environment [21]. The antimicrobial resistance of *C. difficile* zoonotic isolates, which is increasingly reported in studies, according to the One Health approach, poses a serious public health concern [36]. However, only 1 paper related to *C. difficile* presence in the vicinity of a farm was identified but it is worth to mention about the slightly different studies concerning the human carriage of this species. The factors associated with *C. difficile* colonization were investigated in the USA in patients during admission to 5 various hospital units, including the hematology-oncology, solid organ transplant, general medical and intensive care units. The analysis confirmed a well-known factor of a higher risk of *C. difficile* carriage, i.e., previous hospitalisation, but it also found the link with residential proximity to a livestock farm. The colonisation prevalence was the highest in patients admitted to the non-hematology-oncology units with previous hospitalisation, reaching 15.7% and 6.5% in individuals living 1 mile and 50 miles from a livestock farm, respectively. A lower risk, but also on a doubled level, was noticed in patients admitted to the same units but without a history of previous hospitalisation, 10.6% and 4.0%, respectively. However, the authors noticed that this type of relationship between the living environment and *C. difficile* carriage could be caused not only by a higher exposure to *C. difficile* but also by exposure to microbiome disruptors, such as antibiotics and pesticides present in water runoff from the field fertilized by manure [37]. A similar association was indicated also in the USA in the novel approach using GPS, spatial statistics and mixed models. The incidence of community-acquired *C. difficile* infections (i.e., patients who experienced an active infection in the community setting) was higher in individuals living closer to livestock farms and other environmental exposures (proximity to the farming raw materials services and to a nursing home) [38].

The risk of pathogens carriage and microbiome dysbiosis in populations living in livestock farm vicinity might be

associated not only with the elevated levels of zoonotic bacteria present in the residential environment. Pathobionts are the commensal bacteria that become pathogens as a result of complex interactions of the microbial, genetic and host factors that occur as the effect of microbiome dysbiosis. The imbalance of the upper respiratory tract microbiome caused by inhaling the bioaerosol emitted by a livestock farm might reduce the pathobionts containment capacity resulting in the decreasing resistance to colonization [22]. The risk of respiratory tract infections could also be elevated by particulate matter (PM) and endotoxin exposure, harmful agents present in high concentrations in livestock vicinity. The PM and endotoxins inhaled by humans, likely by modulating innate immune responses, might also be a cause of an imbalanced respiratory microbiome, which is supported by animal and *in vitro* experiments [22]. Another possible threat to the human microbiome is environmental exposure to antibiotics and pesticides present in ground water in livestock farm surroundings [37]. Exposure to such disruptors, together with permanent stress generated for community residents by living in an area with a high level of odours [1], may be a significant factor which predisposes these people to an increased susceptibility to acquiring the infection of pathogenic and opportunistic strains [37].

Based on the current state of knowledge, numerous zoonotic strains occur in the livestock environment, posing the health risk for humans, particularly due to antimicrobial resistance. In addition, the probability of the bacteria and genes transmission on populations living in livestock farm vicinity is high. However, the factors associated with both transmission and the actual levels of carriage prevalence in these populations call for global in-depth epidemiological studies to include climate issues and other local conditions. There is an urgent need to focus more studies on LA strains carriage in populations living in various areas, both rural and urban, to compare these

results. Moreover, the evaluation of changes in the human microbiome and their impact on human health needs large epidemiological studies based on populations relocating from urban areas to rural ones with high livestock farm densities. Current knowledge provides the basis for indicating the risk of human colonization via environmental transmission but the impact of LA strains carriage on human health remains unknown.

Limitations

It should be emphasized that the number of studies identified in scientific databases according to the searching criteria defined under this review is very small. Therefore, the strength of the conclusions drawn from the review based on 7 papers is limited. However, current knowledge is a strong justification for the studies on the microbiome of people living in the vicinity of farms to identify the prevalence of zoonotic bacteria in the human microflora. The important question is whether the carriage of zoonotic bacteria, including AR strains and ARGs, in populations living in the livestock surroundings differs from such indicators for the general population. The topic needs to be thoroughly investigated globally, due to the differences in climate, livestock breeding techniques, industrial livestock farming density, antibiotics use in veterinary medicine, human behavior, etc. Nevertheless, conclusions relevant to the aim of the review are presented below.

CONCLUSIONS

The current state of knowledge on the composition of the microflora of humans living in livestock farm vicinity is insufficient to conclude about the microbiome changes caused by the environmental emission of bioaerosol.

Nevertheless, the available studies on the prevalence of the carriage of LA bacteria, including AMR and ARGs, confirm the presence of the zoonotic bacteria in the human microflora in populations without occupational contact with animals.

The review indicates that people living in livestock farm vicinity could be the vectors of zoonotic bacteria with significant importance to public health.

Based on the current state of knowledge, it cannot be ruled out that zoonotic bacteria, as a component of human microflora, have a negative impact on people's health.

Author contributions

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