Distribution of Antibiotic-Resistant Bacteria in the Livestock Farm Environments

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Abstract

The surroundings of livestock farms, including dairy farms, are known to be a major source of development and transmission of antibiotic-resistant bacteria. To control antibiotic-resistant bacteria in the livestock breeding environment, farms have installed livestock wastewater treatment facilities to treat wastewater before discharging the final effluent in nearby rivers or streams. These facilities have been known to serve as hotspots for inter-bacterial antibiotic-resistance gene transfer and extensively antibiotic-resistant bacteria, owing to the accumulation of various antibiotic-resistant bacteria from the livestock breeding environment. This review discusses antibiotic usage in livestock farming, including dairy farms, livestock wastewater treatment plants as hotspots for antibiotic resistant bacteria, and nonenteric gram-negative bacteria from wastewater treatment plants, and previous findings in literature.

Keywords

livestock environment, dairy farm, integron, mcr-1, extensive drug-resistant bacteria

Introduction

Various pathogenic antibiotic-resistant bacteria are being discharged from livestock wastewater treatment plants into its surrounding environments [1–3]. Given continuous antibiotic consumption in livestock is direct driving force for development of antibiotic-resistant bacteria and antibiotic-resistance gene, the significance of antibiotic-resistant bacteria and antibiotic-resistance gene from livestock wastewater treatment plants needs to be evaluated.

Escherichia coli is excellent fecal indicator bacteria to evaluate digestion efficiency of wastewater treatment plants [4,5]. Simultaneously, it can be regarded as bio-indicator for monitoring of antibiotic-resistance gene developed in livestock and farming environment because acquisition of mobile antibiotic-resistance gene by this bacterium is frequently detected [4,5].

Integrons have been considered as transmissible genetic platform mediating resistance to a variety of antibiotics [4]. It is usually associated with antibiotic resistant gram-negative bacteria, especially Enterobacteriaceae such as E. coli, that are originated from mammalian intestine. Mobilized colistin resistance (mcr-1) gene, mobile gene conferring resistance to colistin that is regarded as last option for serious multi-drug resistant bacteria infection is emerging as critical threat to public health [4]. According to a study
in Estonia, mcr-1 carrying E. coli was found in animal waste [6].

*Stenotrophomonas maltophilia* ranked as the third-most prevalent non-enteric gram-negative bacterium associated with hospital-acquired infections, behind *Acinetobacter* spp. and *Pseudomonas aeruginosa* [7]. In addition, *Ochrobactrum anthropic*, the closest bacterium to *Brucella* spp., despite their health of burden is not negligible, information concerning control of Gram-negative bacterium from livestock environment is rarely available [8].

Given that microbial communication in wastewater treatment plants could remarkably encourage transfer of such critical antibiotic-resistance gene to other bacteria, vigorous monitoring antibiotic-resistance genes and antibiotic-resistant bacteria in wastewater treatment plants is vital. Moreover, study on disinfection for control of antibiotic-resistant bacteria should be performed simultaneously.

Therefore, this review paper was organized to provide general information about the above mentioned.

**Antibiotics Usage in Livestock Farming Including Dairy Farm**

Antibiotic usage in livestock farming represents a remarkable portion of the total antibiotic consumption. According to surveys on estimation of antibiotic consumption, more than 70% of overall antibiotic consumption are responsible for livestock farming in both United States of America and Australia [9]. About 50% of overall antibiotic consumption are administered to livestock in China [9].

Antibiotics are necessarily used to produce livestock. However, the misuse and overuse of antibiotics in livestock poses considerable treats to public health in terms of development of antibiotic-resistant bacteria and antibiotic-resistance gene in livestock farming environment, which considerably limiting treatment options for potential human and animal pathogens [1–3]. Scientific communities and international organizations have reported the significance for controlling antibiotic-resistance gene and antibiotic-resistant bacteria from livestock and those environments [1–3]. The use of antibiotics was banned worldwide for growth enhancement and prophylactic treatment in about 2010, only being approved for “therapeutic” purpose with prescription of veterinarian. This regulation was somewhat effective but therapeutic usage of antibiotics is doubled last decade, and consequentially various antibiotic-resistant bacteria is still developed and spread from livestock farming environment to human via direct contact, animal product, and wastewater.

**Livestock Wastewater Treatment Plant: Hot Spot for Antibiotic Resistant Bacteria**

Final effluents from wastewater treatment plants are suspected to be major source of antibiotic-resistance gene and antibiotic-resistant bacteria spread into the environment.
Wastewater treatment plants usually adapted biological treatment “activated sludge process” that consists of microorganism including bacteria, virus, parasite, protozoa, and virus. Organic matters such as phosphorus, nitrous, and other suspended solids from livestock wastewater are removed through food chain of microorganism along the series of treatment processes consisting of solid–liquid separation, anaerobic & aeration digestion, and coagulant sedimentation [11]. During the processes, bacteria from either livestock waste or environment are exposed to antibiotic residue, which would develop antibiotic–resistant bacteria/ antibiotic–resistance gene directly or make a selection pressure for antibiotic–resistant bacteria [11,12]. In addition, antibiotic–resistance gene are transferred through microbial communication from antibiotic-resistant bacteria to non-resistant bacterium [10–12]. In short, wastewater treatment plants make suitable environment for development and spread of antibiotic-resistant bacteria/ antibiotic–resistant bacteria although wastewater is removed effectively.

Non–Enteric Gram–Negative Bacteria from Wasterwater Treatment Plants

To date, antibiotic–resistant bacteria are mostly studied in bacteria focusing on fecal indicator bacteria such as coliforms and enterococci or other pathogenic Entero–bacteriaceae because these bacteria usually are used as bio–indicator for antibiotic resistance profiles in wastewater treatment plants and have more importance in clinical situation [13–15]. Vancomycin resistant Enterococci and Staphylococci. Gram negative bacteria including Salmonella spp., Escherichia coli, Campylobacter spp., Pseudomonad, and Acinetobacter spp. resistant to cephalosporins, carbapenems, and fluoroquinolone has also been addressed in such studies. Although monitoring and characterization of such bacteria is still necessary and valuable, significance of other emerging and environmental origin bacteria, namely Gram–negative bacterium, is not negligible in term of public health. Gram-negative bacterium has been attracted attention in terms of their intrinsic and/or extrinsic extensive drug resistance against carbapenem or colistin, both of which are generally regarded as last resort among antibiotics armamentarium for serious bacterial infection [16]. Gram-negative bacterium raised last decade [16]. Pseudomonas aeruginosa and Acinetobacter spp. (top 2) take up more than 80% of Gram-negative bacterium infection, followed by Stenotrophomonas maltophilia, Aeromonas spp., and Chryseobacterium spp. according to international survey of SENTRY. Although infections except “top 2” have not been reported frequently, their health of burden is also not negligible: S. maltophilia takes up 18%-22% from community acquired septicemia in Taiwan and France [7], and Myroides spp. and Chryseobacterium spp. have been found frequently to be extensively drug–resistant from patient with pneumonia [17,18]. In addition, outbreaks of Elizabethkingia in immune–comprised population was reported from Wisconsin in 2016, in most of which treatment failure was found because of their wide spectrum intrinsic antimicrobial resistance [19]. One of the feasible routes of Gram-negative bacterium infection is wastewater treatment
plant of animal farm where huge amounts of antibiotics are consumed periodically for prevention and/or treatment of bacterial infection in livestock. Notably, activated sludge system is mostly employed as treatment method in wastewater treatment plants because of high efficiency and low energy consuming, which consists of highly various eukaryotes such as protozoa, archaea, bacteria, and fungi, among them bacteria are dominant [20]. Those eukaryotes play as scavenger to digest wastewater and finally devour each other. Recent studies for activated sludge composition analysis using next-generation sequencing revealed that dominant bacteria family are not fecal originated Enterobacteriaceae but Gram-negative bacterium such as Comamonadaceae, Flavobacteriaceae, Pseudomonadaceae, Sphingomonadaceae, and Xanthomonadaceae [20], indicating Gram-negative bacterium could have much chance to be discharged into its receiving water via wastewater treatment plant.

**Summary of the Results Obtained from Previous Studies**

Livestock farms and surrounding environment are widely regarded as possible source for the development and spread of antibiotic resistance bacteria. To control antibiotic-resistant bacteria from livestock environment, wastes containing antibiotic-resistant bacteria are discharged after a series of process in wastewater treatment plant. However, extensive drug resistant bacteria could be developed in wastewater treatment plants by transferring of antibiotic-resistance genes in wastewater treatment plants [21].

Kim [21] isolated total of 125 Gram-negative bacterium isolates, representing 15 genera and 8 families, from final ef fluent of wastewater treatment plant and receiving river in swine, poultry and bovine farms (Table 1). *Stenotrophomonas maltophilia* (16.0%) was most prevalent specie, followed by *Chryseobacterium indologenes* (15.2%), *Stenotrophomonas acidaminiphilia* (10.4%), *Myroides odoratus* (9.6%), and *Serratia marcescens* (8.0%) in Table 1.

Park et al. [22] reported that *Escherichia coli* loads, antibiotic-resistance profiles, antibiotic resistance genes such as integron and *mcr-1* were investigated. In addition, whole genome sequencing was conducted against the plasmid of *mcr-1* positive strain. The *Escherichia coli* loads decreased gradually across the treatment process. However, the proportions of antibiotic resistance and integron carrying isolates were maintained across treatments [21,22]. Of the integron-positive isolates, 17.9% harbored the integron-associated gene cassettes *aadA2, aadA12, aadA22, dfrA15* and *mcr-1*. This is the first description of a class 1 integron containing the *aadA12* gene cassette and *mcr-1* from swine farm in South Korea, reflecting the fact that novel antibiotic-resistance gene cassette arrays could be generated in swine farm wastewater treatment plants [21,22]. Whole genome sequencing analysis of *mcr-1* carrying isolate revealed that it harbored 6 different plasmids and various resistance genes conferring resistance to beta-lactams, sulphonamide, aminoglycoside, trimethoprim, and colistin. In addition, genetic platform (tnpA ISAp1) mediating transfer of *mcr-1* to other bacteria was found in upstream of *mcr-1* [21,22]. These results highlighted the potential risks associated with wastewater discharge from swine farm wastewater treatment plants in terms of the spread of...
Table 1. Distribution of Gram-negative bacterium isolates from livestock wastewater treatment plants and surrounding environment

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>%</th>
<th>Genus &amp; species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>16.0</td>
<td>Stenotrophomonas maltophilia</td>
<td>Xanthomonadaceae</td>
</tr>
<tr>
<td>19</td>
<td>15.2</td>
<td>Chryseobacterium indologenes</td>
<td>Flavobacteriaceae</td>
</tr>
<tr>
<td>13</td>
<td>10.4</td>
<td>Stenotrophomonas acidaminiphila</td>
<td>Xanthomonadaceae</td>
</tr>
<tr>
<td>12</td>
<td>9.6</td>
<td>Myroides odoratus</td>
<td>Flavobacteriaceae</td>
</tr>
<tr>
<td>10</td>
<td>8.0</td>
<td>Serratia marcescens</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>8</td>
<td>6.4</td>
<td>Aeromonas salmonicida</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>Pseudomonas anguilliseptica</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>Pseudomonas putida</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>6</td>
<td>4.8</td>
<td>Aeromonas sobria</td>
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</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>Ralstonia picketti</td>
<td>Ralstoniaceae</td>
</tr>
<tr>
<td>3</td>
<td>2.4</td>
<td>Castellaniella caeni</td>
<td>Alcaligenaceae</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>Aeromonas caviae</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>Pseudomonas fluorescens</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>Ochrobactrum anthropi</td>
<td>Brucellaceae</td>
</tr>
<tr>
<td>2</td>
<td>1.6</td>
<td>Alcaligenes faecalis</td>
<td>Alcaligenaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Achromobacter insolitus</td>
<td>Alcaligenaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Ochrobactrum sp.</td>
<td>Brucellaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Escherichia coli</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Proteus penneri</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Providencia alcalifaciens</td>
<td>Enterobacteriaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Chryseobacterium meningoseptica</td>
<td>Flavobacteriaceae</td>
</tr>
<tr>
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<td>0.8</td>
<td>Chromobacterium violaceum</td>
<td>Neisseriaceae</td>
</tr>
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<td>Pseudomonas protegens</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>Pseudomonas stutzeri</td>
<td>Pseudomonadaceae</td>
</tr>
<tr>
<td>125</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table from Kim with permission of the author [21].

antibiotic-resistant _Escherichia coli_ to the aquatic environment [21,22].

Also, Kim et al. [23] demonstrated that the significance of the presence of _Stenotrophomonas maltophilia_, an emerging extensive drug resistant bacterium, was investigated in final effluents and receiving rivers of pig farm wastewater treatment plants. _Stenotrophomonas maltophilia_ isolates showed extensive drug resistance, i.e., resistant to all classes of antibiotics except 2 classes including quinolones and tetracyclines [21,23]. Moreover, for the first time, it is reported that _Stenotrophomonas maltophilia_ isolates from wastewater treatment plants exhibited strong genetic similarity to clinical isolates causing fatal case, indicating _Stenotrophomonas maltophilia_ discharged from wastewater treatment plants could be translocated to clinical settings [21,23].

In conclusion, Kim [21–23] reported that these results firstly provided the evidence that antibiotic-resistant bacteria carrying diverse resistance genes including _mcr-1_ are being discharged from livestock environment, exhibiting high genetic similarity to clinical isolates. Furthermore, it is urgent to find alternative disinfectants that eradicate antibiotic-resistant bacteria through additional experiments and verification.

Conclusion

Based on research on the pathway of extensively antibiotic-resistant bacteria infection
that can be spread between people, animals, and the environment, solutions that take into account the welfare of humans and animals at the same time are essential. It will also help the harmonious ecosystem environment by taking relevant measures in terms of environment. Environmental characteristics and commonalities should be identified by sharing antibiotic resistance information collected not only in Korea but also abroad. In addition, it is necessary to quickly reduce and resolve the spread of extensively antibiotic-resistant bacteria between people, animals and the environment through international joint research as well as research in Korea. Consequently, the analysis of the One Health approach on the future spread of human-animal-environmental extensively antibiotic-resistant bacteria is urgently needed to identify pathways and mechanisms and also establish blocking techniques.

Conflict of Interest

The authors declare no potential conflict of interest.

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References

7. Chang YT, Lin CY, Chen YH, Hsueh PR. Update on infections caused by Stenotro-
22. Park JH, Kim YJ, Kim B, Seo KH. Spread of multidrug-resistant Escherichia coli har-