



## Review article:

# Inactivation of antibiotic resistance genes (ARGs) in livestock wastes

Yathrib Al-Ubaidy and Amjed Alsultan \*

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Dewaniyah, Iraq

\*Correspondence: [amjed.talib@qu.edu.iq](mailto:amjed.talib@qu.edu.iq); Tel.: +9647716993176

## Abstract

The manure of cattle is containing more than 60 different ARGs with varying resistome different from heard to another. ARGs are associated with bacteria and their prophages and phages, and can be found extracellular on transposable elements and plasmids. Antibiotic resistance Genes in one species of bacteria can be horizontally transferred to another species by one of three defined mechanisms such as conjugation, transformation and transduction. The rapid dissemination of antibiotic resistance genes in this method is becoming medically confrontation to deal with. Animal livestock wastes consider as hotspot for dissemination of ARGs to natural environment. Therefore, treatment of ARGs in environmental elements including livestock waste can decrease abundance and diversity of these genes and protect human protecting human and animal health. However, there no single treatment method has ability to completely inactivation of resistant bacteria or gene in the animal wastes. In this review, we evaluate some technologies that used to inactivate of ARGs in livestock wastes.

**Keyword:** Antibiotic resistance, antibiotic resistant gene (ARGs), dissemination of antibiotic resistant genes.

## 1. Introduction

Antibacterial resistance is more important problem whether in animals or in human (1), global death annually from this trouble estimated 700 000 in 2014 – 10 million by 2050 by cost reached to 100 trillion \$ USD (2). Animals that exposure to large amount of antibiotics, (clinical or agriculture resource), it has led to development of bacteria resistance. Antibiotic resistant bacteria (ARB) can develop of chromosomal DNA mutation (3). This bacteria product varies types of antibiotic resistance genes (ARGs) that can transfer from type of bacteria to other types, pathogen or non-pathogen, by horizontal gene transfer (HGT) via conjunction, transformation, and transduction, ARGs transferred by mobile genetic elements (MGE) integrons, plasmids, and transposon (4). Livestock manure contains many types of (ARGs) that used in soil

fertilization, which lead to separation of these genes in the environment e.g. Soil and water (5, 6, and 7) which mean increasing in morbidity and mortality rate in animals farms, and very high economic losses in the world (8, 9). Human and animals that exposure to ARB via varies routes like inhalation and ingestion (10). According to the WHO in 2017, ARB is the highest danger on the human health, it annually causes about 23 000 deaths in USA deaths and 25 000 in the Europe (4). In this review we highlighted on possibility of treat this problem by some technologies that used to inactivate of ARGs in livestock wastes. many conventional methods are used to degrade ARB and eliminate ARGs from contaminate soil and waste water, like membrane filtration, chlorination, ozone, ultraviolet irradiation (UV), Non-thermal atmospheric pressure



plasmas (NTAPPs) and pulsed electric field (PEF) (3, 11-15).

## **2. Deactivation of ARG:**

### **2.1. Removal of ARGs from livestock waste:**

#### **2.1.1. Conventional methods:**

Livestock waste is contaminating soil with ARB and ARGs when addition to it, therefore, this problem has been treated with some methods that inactivate or decrease the number of these bacteria and genes (16), one of these methods is field treatment system by decreasing nitrogen with Wood chips denitrifying bioreactors, but in this method, manure can retain 70-90% of manure borne antibiotics (17). Another method, it is the best one to removing antibiotics residues from manure and soil, is Bio chars (agricultural residues and forestry) (18) by decreasing the level of zinc and copper and reducing heavy metals in soil (19,20).

#### **2.1.2. Composting:**

Composting or anaerobic digestion (AD) is very important method to removing ARGs from manure (21) including mineralization and mummification of organic component (22, 23). It is an incubation experiment for 120 days (24) the increasing of temperature at composting (44- 65 °C) for a 3 weeks long experiment, gives a good result for decreasing ARGs (25,26). the addition of natural zeolite during composting can be reduce some ARGs and quicken removal of pathogenic bacteria to minimizing environmental risk (27,28). AD is used to settle manure and methane produce as final end product (29). Aerobic composting has been decreased the MGEs and ARGs in cattle manure (24).

## **2.2. Removal of ARGs and ARB from wastewater:**

### **2.2.1. Conventional methods:**

One of the most important sources of ARB and their genes is wastewater. Plant which routinely used to treat wastewater (WWTP) in big cities consider as a reservoir or hotspot of

ARB and ARG and have a critical role in dissemination of ARB& ARG in natural environments (32).

Human activity and Pollutants such heavy metal make animal and human environment as suitable place for dissemination of ARGs by provide good condition for interaction between bacterial species and transmission of ARGs within mobile genetic elements (MGEs) by HGT. Several studies documented that class 1 integron frequently found in WWTP and suggest that plant represent as a risk factor for human and animal lives (33). In many countries, wastewater reuse again as drinking water or for agriculture proposes. many methods were used for treatment of wastewater including biological or physiochemical method. All methods act on remove of chemical contaminants agent, organic matter and microorganism (30). Efficacy of these methods evaluate by measure some harmful reagent and substance such as ammonia, nitrate and coliform bacteria in treated water. unfortunately, many of these method able to inactivate or damage of ARGs. these genes can be extracellular and enter to a new bacteria and make it resistant to antibiotics. A selective pressure occurs when antibiotic residue found in the environment with ARB. The residue permits to grow of ARB and inhibit or kill sensitive one which lead to rapid spread of ARB in natural environments (31). Wide use of water that come from wastewater in human and animal application involve in highly spread of ARGs and represent as an important source of ARB (31)

### **2.2.2. Chlorination:**

in 1980s, studies begin to identify the effect of chlorination on ARB in wastewater was observed (38). Chlorination is the prime disinfection method used in treatment of drinking water because its low cost, its easily to application and capability to inactivate a wide range of bacteria. It is used due to its strong reactions with varies cellular



components and its ability to break bacterial metabolic processes (39). chlorine at high doses (>20 mg/L) can to decrease resistance genes (34) but some ARB have been reported to show resistance to chlorine (40). According to several studies, Chlorination is effected method to inactivate of ARGs followed by UV and Ozone (41).

### 2.2.3. UV radiation:

UV photolysis is most important technology for ARG inactivation at laboratory, based on its effctily used in sterilization, Inactivation ability of UV irradiation was ingested against ARGs (34).

UV irradiation can have affected for both chromosomal and plasmid DNA. The medium presence UV disinfection mechanisms effect on ARGs transfer and conjugation. UV alone has a few impact on the cell membrane, denaturant of the plasmid that carrying ARGs and death of the bacterial cell (36). Chromosomal DNA is resist harmful effect of UV as a compare with plasmid DNA and required high density and more of UV in order to DNA or gene inactivation (34).

the MAR rising at a lower UV flounce (5 mg/cm<sup>2</sup>), also increasing the UV flounce to 10 mJ/cm<sup>2</sup> did not affecting on MAR indictor. It may exposure to a higher UV flounce to decreased the MAR indicator. The effected of UV irradiation in damaging ARGs was obtained at different UV flounces (37)

### 2.2.4. Non-thermal atmospheric pressure plasmas (NTAPPs):

Non-thermal atmospheric pressure plasma (NTAPP) is developed oxidation processes (42). Plasma may be reactive nitrogen and oxygen like H<sub>2</sub>O<sub>2</sub>, ozone, OH, UV photons, nitric oxide, and electrons and ions that act as microorganisms inhepetors (43) that lead to acidification of liquid (PH 2) (49). Plasma is an ionized gas. It results from thermal energy and electric radiation (44, 45). plasma has effect not on ARB and ARGs only, it also

affected on the mechanisms of HGT (3). Energy may transfer to the equal types in gas by clash to release the ionized gas (46). The source of plasma is mostly consisting from dielectric barrier discharges, contact glow discharges, and corona discharges (47, 48). Plasma can inactivate vegetative and spore forms of bacteria (50, 51). There is various plasma discharges can applied to degradation of ARB and their genes, e.g. plasma discharge in and on liquid surface and discharge in bubbles, (3).

### 3. Discussion:

Antibiotic resistance is main problem in animals and human all over the world (1). Continuous exposure to antibiotics in clinic and agriculture uses lead to development of ARB (3) that product varies types of ARGs (4) which is diffuse in environment (5), it is cause high morbidity and mortality in animals farms and high economic losses in the world (8,9).

Scientists find some methods and technologies to elimination or decreasing ARGs (Figure 1). In livestock manure there are conventional methods like removing antibiotics residues from manure and soil by adding Bio chars (18). Composting is very important method to removing ARGs from manure (21).

In wastewater there are many technologies to inactivate ARB and ARGs like membrane filtration, chlorination, ozonation, ultra-violate irradiation(UV), non-thermal atmospheric plasma (NTAPPs), and pulsed electric field (PEF) (3, 11-15). The studies indicated that the effective method for inactivation ARGs is chlorination, followed by UV, then Ozonation (41).

Chlorination is the older technology, affected on ARB (38), it has strong reactions with varies cellular components and it has ability to break bacterial metabolic processes (39).

UV radiation is more important technology for ARGs inactivation (34) in different UV flounces (37).



Figure 1 : Inactivation strategy of Args in farm animal wastes .

## References:

1. Wright, G. D. (2010). Q&A: Antibiotic resistance: where does it come from and what can we do about it?. *BMC biology*, 8(1), 1-6.
2. O'Neill, J. (2014). Review on antimicrobial resistance. *Antimicrobial resistance: tackling a crisis for the health and wealth of nations*, 2014(4).
3. Courti, I., Muja, C., Maho, T., Sainct, F. P., & Guillot, P. (2022). Degradation of Bacterial Antibiotic Resistance Genes during Exposure to Non-Thermal Atmospheric Pressure Plasma. *Antibiotics*, 11(6), 747.
4. Sachdeva, S., Palur, R. V., Sudhakar, K. U., & Rathinavelan, T. (2017). E. coli group 1 capsular polysaccharide exportation nanomachinery as a plausible antivirulence target in the perspective of emerging antimicrobial resistance. *Frontiers in microbiology*, 8, 70.
5. Gullberg, E., Cao, S., Berg, O. G., Ilbäck, C., Sandegren, L., Hughes, D., & Andersson, D. I. (2011). Selection of resistant bacteria at very low antibiotic concentrations. *PLoS pathogens*, 7(7), e1002158.
6. Rysz, M., & Alvarez, P. J. (2004). Amplification and attenuation of tetracycline resistance in soil bacteria: aquifer column experiments. *Water Research*, 38(17), 3705-3712.
7. Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J. M., & Zhang, T. (2015). Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *The ISME journal*, 9(11), 2490-2502.
8. Roberts, R. R., Hota, B., Ahmad, I., & Scott, R. D. (2009). II, Foster SD, Abbasi F, Schabowski S, Kampe LM, Ciavarella GG, Supino M, Naples J, Cordell R, Levy SB, Weinstein RA: Hospital and Societal Costs of Antimicrobial-Resistant Infections in a Chicago Teaching Hospital: Implications for Antibiotic Stewardship. *Clin Infect Dis*, 49, 1175-1184.
9. Christaki, E., Marcou, M., & Tofarides, A. (2020). Antimicrobial resistance in bacteria: mechanisms, evolution, and persistence. *Journal of molecular evolution*, 88(1), 26-40.
10. He, Y., Yuan, Q., Mathieu, J., Stadler, L., Senehi, N., Sun, R., & Alvarez, P. J. (2020). Antibiotic resistance genes from livestock waste: Occurrence, dissemination, and treatment. *NPJ Clean Water*, 3(1), 1-11.
11. Cheng, H., & Hong, P. Y. (2017). Removal of antibiotic-resistant bacteria and antibiotic resistance genes affected by varying degrees of fouling on anaerobic microfiltration membranes. *Environmental science & technology*, 51(21), 12200-12209.
12. Zheng, J., Su, C., Zhou, J., Xu, L., Qian, Y., & Chen, H. (2017). Effects and mechanisms of ultraviolet, chlorination, and ozone disinfection on antibiotic resistance genes in secondary effluents of municipal wastewater treatment plants. *Chemical Engineering Journal*, 317, 309-316.
13. Czekalski, N., Imminger, S., Salhi, E., Veljkovic, M., Kleffel, K., Drissner, D., ... & Von Gunten, U. (2016). Inactivation of antibiotic resistant bacteria and resistance genes by ozone: from laboratory experiments to full-scale wastewater treatment. *Environmental science & technology*, 50(21), 11862-11871.
14. Sousa, J. M., Macedo, G., Pedrosa, M., Becerra-Castro, C., Castro-Silva, S., Pereira, M. F. R., ... & Manaia, C. M. (2017). Ozonation and UV254 nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. *Journal of Hazardous Materials*, 323, 434-441.
15. Furukawa, T., Ueno, T., Matsumura, M., Amarasiri, M., & Sei, K. (2022). Inactivation of antibiotic resistant bacteria and their resistance genes in sewage by applying pulsed electric fields. *Journal of Hazardous Materials*, 424, 127382.
16. Chu, H. C., Hwang, G. J., Tsai, C. C., & Tseng, J. C. (2010). A two-tier test approach to developing location-aware mobile learning systems for natural science courses. *Computers & Education*, 55(4), 1618-1627.
17. Aliev, R. A., Pedrycz, W., Guirimov, B. G., Aliev, R. R., Ilhan, U., Babagil, M., & Mammadli, S. (2011). Type-2 fuzzy neural networks with fuzzy clustering and differential evolution optimization. *Information Sciences*, 181(9), 1591-1608.
18. Frerk, C., Mitchell, V. S., McNarry, A. F., Mendonca, C., Bhagrath, R., Patel, A., ... & Ahmad, I. (2015). Difficult Airway Society 2015 guidelines for management of unanticipated difficult intubation in adults. *BJA: British Journal of Anaesthesia*, 115(6), 827-848.
19. Cui, Y., Li, Y., Wang, Z., Lei, Q., Koizumi, Y., & Chiba, A. (2017). Regulating twin boundary mobility by annealing in magnesium and its alloys. *International Journal of Plasticity*, 99, 1-18.



20. Li, Y., Wang, N., Liu, J., & Hou, X. (2017). Demystifying neural style transfer. arXiv preprint arXiv:1701.01036.
21. Collignon, P. J., & McEwen, S. A. (2019). One health—its importance in helping to better control antimicrobial resistance. *Tropical medicine and infectious disease*, 4(1), 22.
22. Bernal, G., Jiménez-Chafey, M. I., & Domenech Rodríguez, M. M. (2009). Cultural adaptation of treatments: A resource for considering culture in evidence-based practice. *Professional Psychology: Research and Practice*, 40(4), 361.
23. Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., ... & Liang, T. (2020). Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. *bmj*, 369.
24. Li, W., Kong, L., Chen, C., Gou, J., Sheng, S., Zhang, W., ... & Wu, K. (2018). Experimental realization of honeycomb borophene. *Science Bulletin*, 63(5), 282-286.
25. Chou, C. H., Shrestha, S., Yang, C. D., Chang, N. W., Lin, Y. L., Liao, K. W., ... & Huang, H. D. (2018). miRTarBase update 2018: a resource for experimentally validated microRNA-target interactions. *Nucleic acids research*, 46(D1), D296-D302.
26. Gao, S., Tang, G., Hua, D., Xiong, R., Han, J., Jiang, S., ... & Huang, C. (2019). Stimuli-responsive bio-based polymeric systems and their applications. *Journal of Materials Chemistry B*, 7(5), 709-729.
27. Zhang, J., Terrones, M., Park, C. R., Mukherjee, R., Monthieux, M., Koratkar, N., ... & Bianco, A. (2016). Carbon science in 2016: Status, challenges and perspectives. *Carbon*, 98(70), 708-732.
28. Peng, P., Barnes, M., Wang, C., Wang, W., Li, S., Swanson, H. L., ... & Tao, S. (2018). A meta-analysis on the relation between reading and working memory. *Psychological bulletin*, 144(1), 48.
29. Fubin, P., Lei, G., Yubo, Y., Qiangsheng, B., & Jianfei, J. (2016, October). Effects of geometrical parameters on the performance of Rogowski coil for current measuring. In 2016 IEEE PES Asia-Pacific Power and Energy Engineering Conference (APPEEC) (pp. 232-236). IEEE.
30. Hong, Q. N., Fàbregues, S., Bartlett, G., Boardman, F., Cargo, M., Dagenais, P., ... & Pluye, P. (2018). The Mixed Methods Appraisal Tool (MMAT) version 2018 for information professionals and researchers. *Education for information*, 34(4), 285-291.
31. Gao, X., Ren, Y., & Umar, M. (2022). To what extent does COVID-19 drive stock market volatility? A comparison between the US and China. *Economic Research-Ekonomska Istraživanja*, 35(1), 1686-1706.
32. Rizzo, L., Manai, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., ... & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. *Science of the total environment*, 447, 345-360.
33. Cornu, J. N., Ahyai, S., Bachmann, A., de la Rosette, J., Gilling, P., Gratzke, C., ... & Madersbacher, S. (2015). A systematic review and meta-analysis of functional outcomes and complications following transurethral procedures for lower urinary tract symptoms resulting from benign prostatic obstruction: an update. *European urology*, 67(6), 1066-1096.
34. Kena, G., Musu-Gillette, L., Robinson, J., Wang, X., Rathbun, A., Zhang, J., ... & Velez, E. D. V. (2015). The Condition of Education 2015. NCES 2015-144. National Center for Education Statistics.
35. Li, Z., Xiao, C., Yang, Y., Harvey, S. P., Kim, D. H., Christians, J. A., ... & Zhu, K. (2017). Extrinsic ion migration in perovskite solar cells. *Energy & Environmental Science*, 10(5), 1234-1242.
36. Zhou, Z., Jiang, Y., Wang, Z., Gou, Z., Lyu, J., Li, W., ... & Tian, Z. (2015). Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean. *Nature biotechnology*, 33(4), 408-414.
37. Destiani, R., & Templeton, M. R. (2019). Chlorination and ultraviolet disinfection of antibiotic-resistant bacteria and antibiotic resistance genes in drinking water. *AIMS Environmental Science*, 6(3), 222-241.
38. Armstrong, J. S. (1982). The value of formal planning for strategic decisions: Review of empirical research. *Strategic management journal*, 3(3), 197-211.
39. Virto, R., Manas, P., Alvarez, I., Condon, S., & Raso, J. (2005). Membrane damage and microbial inactivation by chlorine in the absence and presence of a chlorine-demanding substrate. *Applied and environmental microbiology*, 71(9), 5022-5028.
40. Zhao, D., Huang, Z., Umino, N., Hasegawa, A., & Kanamori, H. (2011). Structural heterogeneity in the megathrust zone and mechanism of the 2011 Tohoku-oki earthquake (Mw 9.0). *Geophysical Research Letters*, 38(17).
41. Stange, C., Sidhu, J. P. S., Toze, S., & Tiehm, A. (2019). Comparative removal of antibiotic resistance genes during chlorination, ozonation, and UV treatment. *International journal of hygiene and environmental health*, 222(3), 541-548.
42. Liao, X., Cullen, P. J., Liu, D., Muhammad, A. I., Chen, S., Ye, X., ... & Ding, T. (2018). Combating



- Staphylococcus aureus and its methicillin resistance gene (mecA) with cold plasma. *Science of the Total Environment*, 645, 1287-1295.
43. Barjasteh, A., Dehghani, Z., Lamichhane, P., Kaushik, N., Choi, E. H., & Kaushik, N. K. (2021). Recent progress in applications of non-thermal plasma for water purification, bio-sterilization, and decontamination. *Applied Sciences*, 11(8), 3372.
  44. Rezaei, F., Vanraes, P., Nikiforov, A., Morent, R., & De Geyter, N. (2019). Applications of plasma-liquid systems: A review. *Materials*, 12(17), 2751.
  45. Zeghioud, H., Nguyen-Tri, P., Khezami, L., Amrane, A., & Assadi, A. A. (2020). Review on discharge Plasma for water treatment: mechanism, reactor geometries, active species and combined processes. *Journal of Water Process Engineering*, 38, 101664.
  46. Kim, S., & Kim, C. H. (2021). Applications of plasma-activated liquid in the medical field. *Biomedicines*, 9(11), 1700.
  47. Jiang, B., Zheng, J., Qiu, S., Wu, M., Zhang, Q., Yan, Z., & Xue, Q. (2014). Review on electrical discharge plasma technology for wastewater remediation. *Chemical Engineering Journal*, 236, 348-368.
  48. Foster, J. E. (2017). Plasma-based water purification: Challenges and prospects for the future. *Physics of Plasmas*, 24(5), 055501.
  49. Bruggeman, P., & Leys, C. (2009). Non-thermal plasmas in and in contact with liquids. *Journal of Physics D: Applied Physics*, 42(5), 053001.
  50. Yang, Y., Wan, K., Yang, Z., Li, D., Li, G., Zhang, S., ... & Yu, X. (2020). Inactivation of antibiotic resistant *Escherichia coli* and degradation of its resistance genes by glow discharge plasma in an aqueous solution. *Chemosphere*, 252, 126476.
  51. Liao, X., Liu, D., & Ding, T. (2020). Nonthermal plasma induces the viable-but-nonculturable state in *Staphylococcus aureus* via metabolic suppression and the oxidative stress response. *Applied and Environmental Microbiology*, 86(5), e02216-19.