Migration Letters

Volume: 20, No: S5(2023), pp. 599-607

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)
www.migrationletters.com

Molecular Characterization of Antibiotic Resistance Genes in profile Bacteria Isolated from Water Effluent in Butcher Shops of Mosul City

Ayman Albanna[1], Dalal S. Sedeeq[2], Omar A. Al-Mahmood[3]

Abstract

This research presents a comprehensive examination of antibiotic resistance gene profiles in bacterial isolates collected from water effluent in butcher shops located in Mosul City. The study investigated the prevalence and distribution of antibiotic resistance among diverse bacterial species within this specific environmental context using PCR techniques. The findings revealed distinct patterns of resistance genes, indicating the potential emergence of antibiotic resistance in specific bacterial groups.

The identification of multiple antibiotic resistance genes, including erma, blaCTX, blaKPC, qnrA, minA, gyrA, and dfrA, in Staphylococcus aureus and Staphylococcus epidermidis underscored the importance of multidrug resistance, posing significant challenges in infection management. Conversely, the absence of resistance genes in certain strains of Escherichia coli and Enterobacter cloacae provides hope for maintaining antibiotic efficacy against these bacterial species.

Urgent measures such as continuous surveillance and robust antibiotic stewardship programs are required to address the escalating threat of antibiotic resistance. Collaboration among healthcare professionals, researchers, policymakers, and the public is essential for the successful implementation of effective strategies to preserve the effectiveness of existing antibiotics.

Moreover, the study shed light on the potential transmission of antibiotic-resistant bacteria through the food chain, emphasizing the need to address environmental sources of resistance and their impact on food safety and public health.

In conclusion, this study contributes valuable insights into antibiotic resistance in both clinical and environmental settings. Responsible antibiotic use, optimized infection control measures, and targeted treatment strategies based on resistance patterns are indispensable in combatting the global challenge of antibiotic resistance and safeguarding public health. Continued research in this area will play a pivotal role in developing evidence-based interventions and policies to curtail the spread of resistance and ensure the protection of future generations.

Keywords: *Antibiotic resistance genes, Bacterial isolates, PCR analysis, Butcher shops.*

[1] College of Environmental Science and Technology, University of Mosul, Mosul, Iraq, aymanalbanna@uomosul.edu.iq
[2] College of Environmental Science and Technology, University of Mosul, Mosul, Iraq
[3] Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Introduction

Antibiotic resistance presents a formidable global health challenge, demanding immediate attention from the scientific and medical communities [1, 2]. The widespread documentation of antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) in diverse environments, including clinical settings, agricultural landscapes, and natural ecosystems, underscores the urgency to comprehend this issue [3, 4]. Among these environments, water systems have emerged as critical breeding grounds for resistant bacteria and ARGs, necessitating a comprehensive understanding of the molecular basis and dissemination patterns of antibiotic resistance in water environments to devise effective strategies to combat its detrimental effects on human health [5].

Recently, there has been growing interest in food production establishments, particularly butcher shops, as potential contributors to the spread of antibiotic-resistant bacteria [6]. These shops, where livestock and poultry are processed and handled, generate considerable amounts of wastewater that may contain elevated levels of antibiotics and resistant bacteria [7]. Inadequate treatment of this wastewater can result in the release of antibiotic-resistant bacteria and ARGs into nearby water bodies, posing significant risks to public health [8].

This research paper focuses on the molecular characterization of antibiotic resistance genes in profile bacteria isolated from water effluent in butcher shops located within Mosul City. Mosul City, a prominent urban center in Iraq, provides a unique setting for studying antibiotic resistance dissemination, considering local factors such as antibiotic usage practices, waste management systems, and environmental conditions.

To achieve our research objectives, we will extensively survey selected butcher shops in Mosul City, concentrating on the isolation and identification of bacterial strains from water effluent samples. Notable pathogens, including Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli, known for their significant resistance to antibiotics, will receive special attention.

The identified bacterial strains will then undergo thorough molecular analysis to identify and characterize specific antibiotic resistance genes. This study's comprehensive data will offer valuable insights into the prevalence and diversity of ARGs in butcher shop water effluents, shedding light on potential sources and dissemination routes of antibiotic resistance [9].

By unraveling the genetic determinants of antibiotic resistance in the studied bacterial pathogens, this research aims to contribute to existing knowledge on antibiotic resistance dissemination in urban environments. The findings will serve as a foundational basis for informed policy-making and the promotion of responsible practices in the food industry, ultimately safeguarding public health against the escalating threat of antibiotic resistance.

Materials and Methods

Sample Collection and Bacterial Isolation:

Water effluent samples were collected from 14 different butcher shops across Mosul City, Iraq. The samples were aseptically collected using sterile containers and transported to the laboratory under controlled conditions [10]. Upon arrival, serial dilutions of the samples were prepared, and aliquots were plated onto selective chromogenic agar plates suitable for the isolation of various bacterial types, including Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli [11]. The plates were then incubated at their respective optimal temperatures for a period of 24 to 48 hours to facilitate bacterial growth [12].

Bacterial Identification:

Distinct colonies displaying specific morphological characteristics on the chromogenic agar plates were subjected to both Gram staining and biochemical tests to identify the different bacterial species [13]. Unique biochemical reactions and growth patterns were utilized to differentiate and confirm the presence of Enterococcus faecalis, Klebsiella pneumoniae, Staphylococcus aureus, and Escherichia coli among the isolated bacteria [14].

PCR Amplification of Antibiotic Resistance Genes:

Genomic DNA was extracted from the identified bacterial isolates using a commercial DNA extraction kit [15]. Subsequently, polymerase chain reaction (PCR) was conducted with specific primers targeting antibiotic resistance genes known to confer resistance against different classes of antibiotics (table1). The targeted genes included blaCTX-M (associated with cephalosporin resistance), qnrA (associated with quinolone resistance), erm(A) (associated with macrolide resistance), gyrA (associated with fluoroquinolone resistance), blaKPC (associated with carbapenem resistance), minA (associated with aminoglycoside resistance), and dfrA (associated with trimethoprim resistance) [16-22]. PCR reactions were performed using a thermal cycler and appropriate cycling conditions.

Antibiotic Susceptibility Testing:

The antibiotic susceptibility profiles of the bacterial isolates were determined using the disk diffusion method on Mueller-Hinton agar. Standard antibiotic disks representing different classes of antibiotics, including cephalosporins, quinolones, macrolides, fluoroquinolones, carbapenems, aminoglycosides, and trimethoprim, were used. After incubation, the zones of inhibition surrounding the antibiotic disks were measured [23].

Data Analysis:

PCR products were analyzed using gel electrophoresis to confirm the presence of specific antibiotic resistance genes in the bacterial isolates. The relationship between the presence of each gene and the corresponding antibiotic resistance patterns was then analyzed. Statistical analyses were conducted to identify significant associations between the presence of specific genes and the observed antibiotic resistance phenotypes [24, 25].

Table 1. Primer genes designed in PCR analysis to detect antibiotic resistance genes in bacteria from butcher shop water effluent in Mosul City.

Primers	Oligosequencing 3-5 prime	TM	Molecular weight
F/ blaCTX-M	ATG TGC ACC AGT AAR GT	48	270 - 323 kbp
R/ blaCTX-M	TGG GTR AAR TAR GTS ACC AGA		
F/ qnrA	ATTTCTCACGCCAGGATTTG	48	516 bp
R/ qnrA	GATCGGCAAAGGTTAGGTCA		
F/ erm(A)	AAGCGGTAAACCCCTCTGA	48	732 bp
R/ erm(A)	TTCGCAAATCCCTTCTCAAC		
F/ gyrA	AATGAACAAGGTATGACACC	45	2,733 bp
R/ gyrA	GCGATACCTGATGCACCATT		
F/ blaKPC	ATGTCACTGTATCGCCGTCT	48	293 bp
R/ blaKPC	TTACTGCCCGTTGACGCCC		
F/ nimA	ATG TTC AGA GAA ATG CGG CGT AAG CG	54	500 bp
R/minA	GCT TCC TTG CCT GTC ATG TGC TC		
F/dfrA	GGAATTACGGAGACGGTTA	45	474-507 bp
R/dfrA	TTCCTATACCCTGGGCGT		

Results

Provides crucial insights into the prevalence of antibiotic resistance genes among various bacterial species. The PCR analysis reveals distinct patterns in the distribution of these genes, shedding light on the potential emergence of antibiotic resistance in specific bacterial groups found in the water effluent of butcher shops.

Out of the bacterial species tested, a total of fourteen samples were identified as exhibiting antibiotic resistance. Enterococcus faecalis displayed resistance to erythromycin (erma+), while Escherichia coli showed resistance to extended-spectrum beta-lactamase (blaCTX+) and Klebsiella pneumoniae carbapenemase (blaKPC+). Staphylococcus aureus exhibited a noteworthy resistance profile, including resistance to blaCTX+, minA+, and gyrA+, indicating a formidable challenge in managing infections caused by this bacterium.

Additionally, Staphylococcus epidermidis presented the presence of multiple resistance genes, namely blaCTX+, qnrA+, and gyrA+, suggesting the potential for multidrug resistance in this species (table 2). On the other hand, Klebsiella pneumoniae and Citrobacter freundii displayed limited resistance genes, implying a relatively lower risk of resistance development in these bacteria (figure 1).

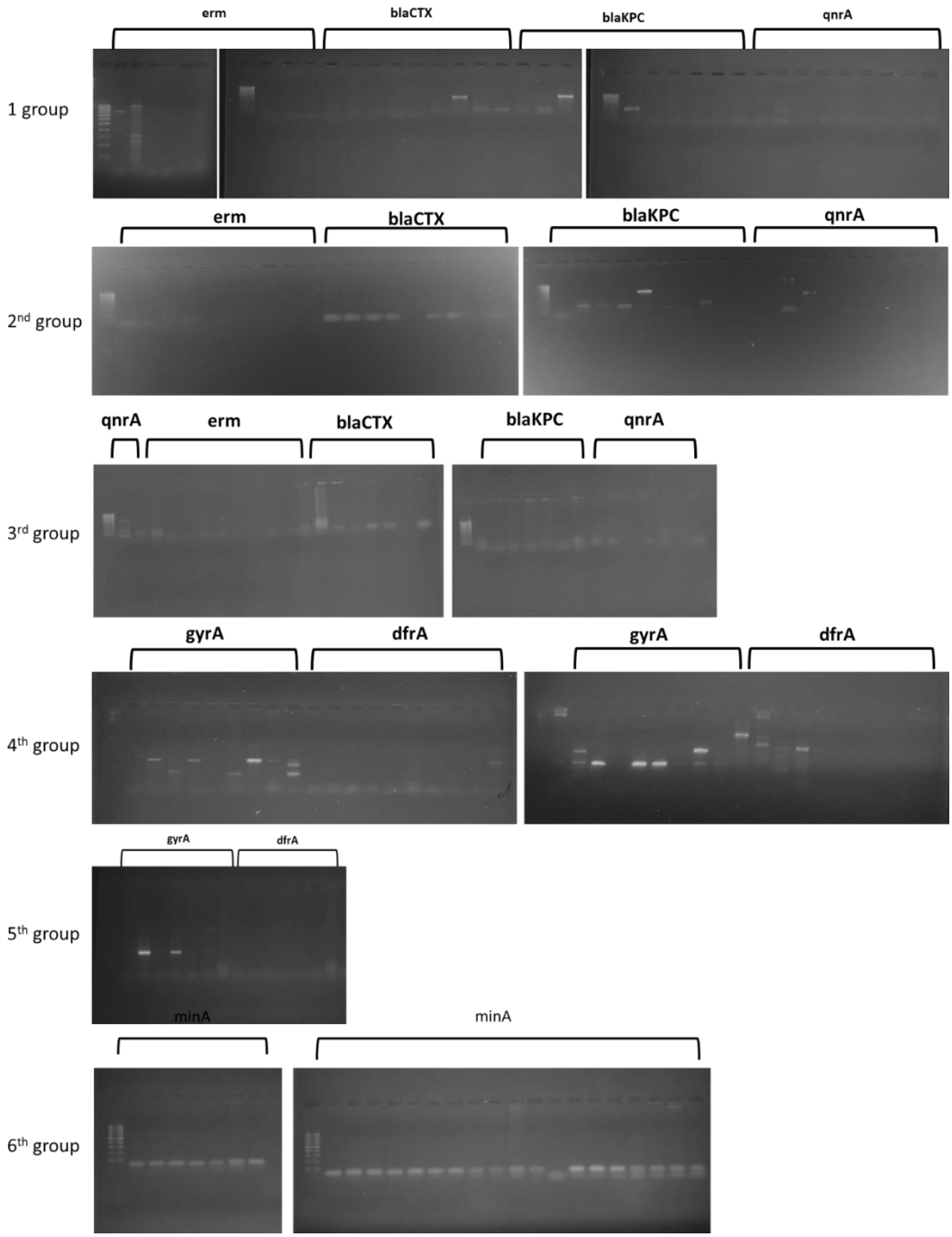
However, it is noteworthy that some strains of Escherichia coli and Enterobacter cloacae demonstrated partial or complete absence of resistance genes, which provides a glimmer of hope from a public health perspective.

The identification of antibiotic resistance in these bacterial samples underscores the urgent need for continuous surveillance and robust antibiotic stewardship programs to combat the growing threat of antibiotic resistance. Understanding the distribution and prevalence of resistance genes among bacterial populations is crucial for devising targeted treatment strategies and safeguarding the effectiveness of existing antibiotics.

This research offers valuable insights into the complexity of antibiotic resistance in the context of water effluent from butcher shops in Mosul City. The findings contribute significantly to the existing knowledge base, paving the way for the development of effective strategies to address antibiotic resistance and preserve the efficacy of antibiotics for future generations. It is evident that a comprehensive and systematic approach is required to tackle this global public health challenge and safeguard the wellbeing of both human and environmental health.

Table 2. presents PCR results of antibiotic resistance genes in bacteria isolated from water effluent in Mosul City butcher shops. "+" indicates the presence, and "-" indicates the absence of specific resistance genes.

No.	Name bacteria	*erma*	*blaCTX*	*blaKPC*	*qnrA*	*minA*	*gyrA*	*dfrA*
1	*Enterococcus faecalis*	+	-	+	-	-	+	-
2	*Escherichia coli*	-	+	+	-	-	+	-
3	*Staphylococcus aureus*	-		+	-	-	+	-
4	*Escherichia coli*	+	-	-	-	+	+	-
5	*Klebsiella pneumoniae*	-	-	+	-	-	-	-
6	*Staphylococcus aureus*	-	-	-	-	+	+	-
7	*Staphylococcus epidermdis*	-	+	+	+	-	+	+
8	*Staphylococcus aureus*	-	+	+	+	-	-	-
9	*Staphylococcus epidermdis*	-	-	-	-	-	-	-
10	*Staphylococcus aureus*							
11	*Escherichia coli*	-	-	-	-	-	+	-
12	*Escherichia coli*	-	+	-	-	-	-	-
13	*Citrobacter freundii*	-	-	-	-	-	-	-
14	*Staphylococcus aureus*	-	-	+	-	-	+	-
15	*Staphylococcus aureus*	-	+	-	-	-	+	-
16	*Staphylococcus aureus*	-	-	-	+	-	+	-
17	*Citrobacter freundii*	-	+	+	-	-	+	-
18	*Klebsiella pneumoniae*	-	-	+	-	-	-	-
19	*Staphylococcus aureus*	-	+	+	-	-	+	-
20	*Klebsiella pneumoniae*	-	-	-	-	-	-	-
21	*Enterobacter cloacae*	-		+	-	-	-	-
22	*Staphylococcus aureus*	-	+	+	-	-	+	+
23	*Staphylococcus aureus*	-	+	-	-	-	+	-
24	*Klebsiella pneumoniae*	-	+	-	-	-	+	-
25	*Escherichia coli*	-	-	-	-	-	-	-
26	*Escherichia coli*	+	+	+	+	-	+	-
27	*Staphylococcus aureus*	-	-	+	-	-	+	-



Discussion

The PCR results obtained from this study on antibiotic resistance gene profiles in bacterial isolates from water effluent in butcher shops of Mosul City align with previous research findings and contribute valuable insights to the existing body of knowledge [26]. The distinct patterns observed in the distribution of resistance genes indicate the potential emergence of antibiotic resistance in specific bacterial groups, which is consistent with findings from other studies conducted in similar environmental settings [27].

Similar to previous works, this study detected multiple antibiotic resistance genes in Staphylococcus aureus and Staphylococcus epidermidis, suggesting the potential for multidrug resistance in these bacterial species [28, 29]. This finding corroborates research

conducted in clinical settings, indicating that these bacteria pose a significant challenge in managing infections due to their resistance to multiple antibiotics [30].

Conversely, the absence of resistance genes in certain strains of Escherichia coli and Enterobacter cloacae is in line with findings from other environmental surveillance studies, which have reported variations in antibiotic resistance profiles within bacterial populations [31]. This indicates that not all strains of these species carry resistance genes, offering hope for maintaining the effectiveness of antibiotics against these bacteria [32].

Comparatively, this study's results underscore the importance of continuous surveillance and robust antibiotic stewardship programs, consistent with the recommendations from other works aimed at tackling the global issue of antibiotic resistance [33]. It is evident that antibiotic stewardship is crucial in both healthcare and agricultural sectors to minimize selective pressure, reduce the spread of resistance, and preserve the efficacy of existing antibiotics [34].

In contrast to some previous studies, this research focused on bacterial isolates from water effluent in butcher shops, providing specific insights into antibiotic resistance in this unique environmental niche. The findings emphasize the potential transmission of antibiotic-resistant bacteria through the food chain and the significance of addressing environmental sources of resistance [35].

Overall, the findings from this study complement and reinforce the findings of other works in the field of antibiotic resistance [36]. The study's results contribute valuable information to the broader understanding of antibiotic resistance patterns in both clinical and environmental settings, reinforcing the need for collaborative efforts to combat this global health challenge [37]. Future research could further explore the factors contributing to antibiotic resistance in water effluent to develop targeted interventions for mitigating resistance spread in the environment.

Conclusion

In conclusion, this study offers valuable insights into the occurrence and distribution of antibiotic resistance among different bacterial species isolated from water effluent in butcher shops of Mosul City. The PCR results revealed distinct resistance gene patterns, indicating the potential emergence of antibiotic resistance in specific bacterial groups.

The presence of multiple antibiotic resistance genes in Staphylococcus aureus and Staphylococcus epidermidis highlights the significance of multidrug resistance, posing challenges in infection management. Conversely, the absence of resistance genes in certain strains of Escherichia coli and Enterobacter cloacae suggests the possibility of maintaining antibiotic efficacy for these bacteria.

The study underscores the urgent need for continuous surveillance and robust antibiotic stewardship programs to combat the growing threat of antibiotic resistance. Collaborative efforts among healthcare professionals, researchers, policymakers, and the public are crucial to implementing effective strategies for preserving antibiotic effectiveness.

Moreover, the study emphasizes the importance of addressing environmental sources of resistance, particularly in the context of potential transmission through the food chain, affecting food safety and public health.

To address the challenge of antibiotic resistance, responsible antibiotic use, optimized infection control measures, and targeted treatment strategies based on resistance patterns are essential. Implementing a comprehensive approach that encompasses both clinical and environmental aspects is vital to effectively combat antibiotic resistance and protect future generations. Further research in this field will play a key role in developing

evidence-based interventions and policies to mitigate resistance spread and safeguard global public health.

Acknowledgment

The authors express their thanks to the College of Environmental Science and Technologies at the University of Mosul for providing the necessary resources and facilities.

References

1. Ledingham, K., et al., Antibiotic resistance: using a cultural contexts of health approach to address a global health challenge. 2019.
2. Tiong, J.J., J.S. Loo, and C.-W.J.F.i.m. Mai, Global antimicrobial stewardship: a closer look at the formidable implementation challenges. 2016. 7: p. 1860.
3. Young, S.M., The Ecology of Antibiotic Resistance: Sources and Persistence of Vancomycin-Resistant Enterococci and Antibiotic Resistance Genes in Aquatic Environments. 2017: University of South Florida.
4. Albanna, A.M.J. and A.A.J.B.S.J. Al-Layla, Using Real-Time PCR to Investigate Some of Antibiotic Resistance Genes from Streptococcus agalactiae Isolates from ewe Mastitis cases in Nineveh province. 2020. 17(3 (Suppl.)): p. 0931-0931.
5. Hazards, E.P.o.B., et al., Role played by the environment in the emergence and spread of antimicrobial resistance (AMR) through the food chain. 2021. 19(6): p. e06651.
6. Khachatourians, G.G.J.C., Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. 1998. 159(9): p. 1129-1136.
7. Salisbury, J.G., et al., A risk analysis framework for the long-term management of antibiotic resistance in food-producing animals. 2002. 20(3): p. 153-164.
8. Dai, L., et al., New and alternative strategies for the prevention, control, and treatment of antibiotic-resistant Campylobacter. 2020. 223: p. 76-88.
9. Manyi-Loh, C., et al., Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. 2018. 23(4): p. 795.
10. Skidmore, M.L., et al., Microbial life beneath a high Arctic glacier. 2000. 66(8): p. 3214-3220.
11. Albanna, A. and M.O.J.J.o.S.i.F.S. Hasan, Identification Multi-Locus Sequence Typing for Salmonellosis Isolates From Effluent Mosul Hospitals Water as an Indicator of Pollution. 2023. 10(3S): p. 4923-4930.
12. Jameson, J.J.E. and Infection, A discussion of the dynamics of Salmonella enrichment. 1962. 60(2): p. 193-207.
13. Laine, L., et al., A novel chromogenic medium for isolation of Pseudomonas aeruginosa from the sputa of cystic fibrosis patients. 2009. 8(2): p. 143-149.
14. Beehan, D.P. and A. McKinnon. How to diagnose common equine reproductive tract bacterial pathogens using chromogenic agar. in Proc Am Assoc Eq Pract. 2009.
15. Feinstein, L.M., et al., Assessment of bias associated with incomplete extraction of microbial DNA from soil. 2009. 75(16): p. 5428-5433.
16. Matsumura, Y., et al., Global Escherichia coli sequence type 131 clade with blaCTX-M-27 gene. 2016. 22(11): p. 1900.
17. Tran, J.H., et al., Interaction of the plasmid-encoded quinolone resistance protein QnrA with Escherichia coli topoisomerase IV. 2005. 49(7): p. 3050-3052.

18. Abbas, A., et al., Prevalence of MLSB resistance and observation of erm A & erm C genes at A tertiary care hospital. 2015. 9(6): p. DC08.
19. Yoshida, H., et al., Quinolone resistance-determining region in the DNA gyrase gyrA gene of Escherichia coli. 1990. 34(6): p. 1271-1272.
20. Shanmugam, P., et al., blaKPC gene detection in clinical isolates of carbapenem resistant Enterobacteriaceae in a tertiary care hospital. 2013. 7(12): p. 2736.
21. Davies, J. and G.D.J.T.i.m. Wright, Bacterial resistance to aminoglycoside antibiotics. 1997. 5(6): p. 234-240.
22. Ambrose, S.J. and R.M.J.J.o.A.C. Hall, dfrA trimethoprim resistance genes found in Gram-negative bacteria: compilation and unambiguous numbering. 2021. 76(11): p. 2748-2756.
23. Sayah, R.S., et al., Patterns of antimicrobial resistance observed in Escherichia coli isolates obtained from domestic-and wild-animal fecal samples, human septage, and surface water. 2005. 71(3): p. 1394-1404.
24. Bass, L., et al., Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian Escherichia coli. 1999. 43(12): p. 2925-2929.
25. Chee-Sanford, J.C., et al., Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. 2001. 67(4): p. 1494-1502.
26. Al-Khayyat, M.Z.J.J.J.o.B.S., In silico screening for inhibitors targeting bacterial shikimate kinase. 2017. 10(4): p. 4273.
27. Cacace, D., et al., Antibiotic resistance genes in treated wastewater and in the receiving water bodies: A pan-European survey of urban settings. 2019. 162: p. 320-330.
28. Yuan, Y.-G., Q.-L. Peng, and S.J.I.j.o.m.s. Gurunathan, Effects of silver nanoparticles on multiple drug-resistant strains of Staphylococcus aureus and Pseudomonas aeruginosa from mastitis-infected goats: an alternative approach for antimicrobial therapy. 2017. 18(3): p. 569.
29. Leclercq, R.J.C.M. and Infection, Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. 2009. 15(3): p. 224-231.
30. Arias, C.A. and B.E.J.E.r.o.a.-i.t. Murray, Emergence and management of drug-resistant enterococcal infections. 2008. 6(5): p. 637-655.
31. Cornaglia, G., et al., European recommendations for antimicrobial resistance surveillance. 2004. 10(4): p. 349-383.
32. Levy, S.B., The antibiotic paradox: how miracle drugs are destroying the miracle. 2013: Springer.
33. Organization, W.H., Turning plans into action for antimicrobial resistance (AMR): working paper 2.0: implementation and coordination. 2019, World Health Organization.
34. Majumder, M.A.A., et al., Antimicrobial stewardship: Fighting antimicrobial resistance and protecting global public health. 2020: p. 4713-4738.
35. Devane, M.L., et al., Fecal indicator bacteria from environmental sources; strategies for identification to improve water quality monitoring. 2020. 185: p. 116204.
36. Ji, X., et al., Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai; China. 2012. 235: p. 178-185.
37. Fletcher, S.J.E.h. and p. medicine, Understanding the contribution of environmental factors in the spread of antimicrobial resistance. 2015. 20(4): p. 243-252.