



*Journal of Advances and  
Scholarly Researches in  
Allied Education*

*Vol. IX, Issue No. XVIII,  
April-2015, ISSN 2230-7540*

**STUDY OF GENES OF ANTIBIOTIC BACTERIA  
DRAINEGE WATER OF MUZAFFERPUR**

AN  
INTERNATIONALLY  
INDEXED PEER  
REVIEWED &  
REFEREED JOURNAL

# Study of Genes of Antibiotic Bacteria Drainage Water of Muzaffarpur

Dr. Nitin Kumar\*

Assistant Professor, Department of Chemistry, Jai Murat Rai Degree College, Nirpur, Patepur, Vaishali

**Abstract** – In this the study of genes of antibiotic bacteria drainage water were studied. The study is conducted in the area of Muzaffarpur. Biofilms were explored utilizing enterococci, staphylococci, enterobacteriaceae, and heterotrophic bacteria as pointer living beings taking into account the developing enthusiasm for the potential pretended by clinic and metropolitan wastewater frameworks in the choice of antibiotic-safe bacteria. Notwithstanding wastewater, biofilms were likewise examined to gauge the event of safe bacteria and their obstruction genes in drinking water from stream bank filtrate, subsequently demonstrating conceivable exchange from wastewater and surface water to the dispersion organization of drinking water. Antibiograms were portrayed by vancomycin-safe enterococci, and subatomic science strategies, including PCR, recognized the van a obstruction quality. Without enterococci, the van a quality was found in squander water biofilms as well as in drinking water biofilms, demonstrating conceivable quality exchange to autochthonous drinking water bacteria. In clinic squander water biofilms, yet in no other compartment, the mec a quality encoding methicillin obstruction in staphylococci was recognized. PCR from Drainage Water Biofilms intensified enterobacterial ampC opposition genes encoding L-lactamase exercises.

**Keywords:** Genes, Antibiotic Bacteria, Drainage Water, Muzaffarpur

-----X-----

## INTRODUCTION

In the treatment of different irresistible sicknesses, specifically, the runs, pneumonia, urinary parcel contaminations, jungle fever, tuberculosis and HIV/AIDS, antibiotics are required. The current worldwide antibiotic-safe bacteria plague, notwithstanding, represents a genuine wellbeing danger. To assess the real worldwide weight of antimicrobial obstruction, different investigations have been completed around the world. Antibiotic-safe bacteria are liable for the passings of more than 25,000 patients for every year in Europe and cost in any event EUR 1.5 billion every year. 63,000 passings happen every year in the United States because of antibiotic-safe bacteria, costing the US wellbeing framework between \$21 billion and \$34 billion yearly (1). Moreover, in agrarian practices and in poultry, antibiotic gatherings, for example, cephalosporins and fluoroquinolone are normally utilized worldwide as development advertisers in sub therapeutic portions and for the avoidance and treatment of diseases (2).

Antibiotic obstruction is a genuine human medical issue that endangers the adequacy of current antibiotic therapy for bacterial diseases such are reality undermining (3). More than 25,000 individuals are assessed to kick the bucket every year in Europe because of diseases brought about by antibiotic-safe bacteria (4). The gut micro biota of people and

creatures is a significant supply of antibiotic-safe Gram-adverse bacteria, especially after presentation to antibiotics. Most antibiotic obstruction genes (ARGs) are found on portable hereditary components, for example, transposons of plasmids and integrons, which can be moved by level quality exchange (HGT) between various bacterial species. Antibiotic-safe bacteria and ARGs have gotten far reaching in common habitats because of the extreme utilization of antimicrobial mixes for clinical and rural purposes. A few late investigations have featured the event of ARGs in non-clinical conditions. The majority of these investigations have zeroed in on profoundly defiled drug industry-related conditions and other anthropogenic exercises. Moreover, antibiotic opposition the study of disease transmission has to a great extent been restricted to clinical settings, however it is turning out to be certain that antibiotic obstruction ought to be checked in the regular habitat for a more far reaching comprehension of the commonness, conveyance, and transmission of ARGs (5)

Since the primary utilization of sulfonamides and penicillin in the last part of the 1930s and mid 1940s for the treatment of human patients with bacterial contaminations, bacterial antibiotic opposition has been a known clinical issue. From that point forward, the utilization of antibiotics in creature horticulture has expanded in amounts that have outperformed the

amount of antibiotics utilized in human clinical conditions. To improve feed productivity and advance development advancement in animals, subtherapeutic portions of antibiotics have been utilized. The 2017 FDA administering thought that it was unlawful to utilize human-related antibiotics for creature development advancement purposes in the United States; however the utilization of different antibiotics in animal's creation to ensure creature wellbeing has proceeded.

The utilization of antibiotics can likewise quicken the advancement of antibiotic opposition genes (ARGs) and bacteria that relieve wellbeing dangers to people and creatures, notwithstanding synthetic contamination brought about by the antibiotics themselves. Such bacteria can be communicated by means of immediate or circuitous contact from the climate to people. Given the developing proof that clinical obstruction is firmly connected with ecological ARGs and bacteria (6), it is very evident that non-pathogenic or natural microorganisms should be remembered for research exercises. There are at present various distributions in various water conditions identifying with the event of ARGs, yet barely any surveys have been finished. This paper gives a review of the most recent writing accessible on the sorts, appropriations, and level exchange of ARGs in various oceanic conditions, just as the atomic strategies used to identify ARGs in the climate.

## METHODOLOGY:

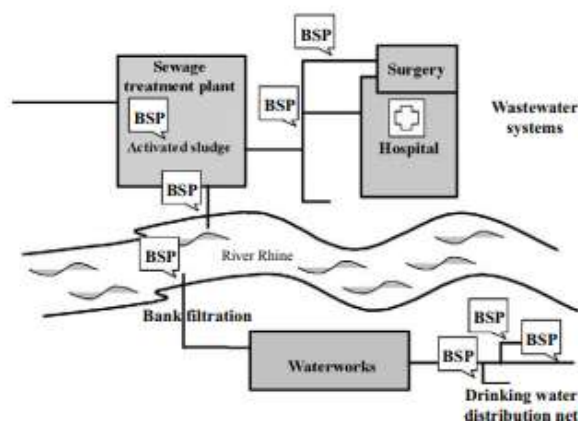
### Study area:

The study of genes of antibiotic bacteria drainage water were is conducted in the area of Muzaffarpur

### Sampling points

Altered Robbins gadgets were introduced at different inspecting focuses inside the water dispersion arrangement of the city of Mainz so as to explore characteristic biofilms in waste water frameworks. In the family branch connections of the appropriation framework, three gadgets were introduced. These inspecting focuses were provided from the waterworks with bank-sifted drinking water and were a good ways off of  $1 \wedge 2$  km from the plant. The progression of water was roughly  $6.0 \text{ O } 0.5 \text{ m}^3/\text{day}^{31}$ . The sanitization was brought out at the waterworks through UV illumination. In the wastewater arrangement of an emergency clinic in the city of Mainz, two examining focuses were organized, one downstream from the careful division and the other at the outlet of the clinical wastewater framework, near the public sewer framework. In the city sewage framework, two biofilm examining focuses were found, one in the natural muck offices and the other at the wastewater treatment plant outlet. Steel platelets were brooded a short separation upstream of the bank filtration in the Rhine River to examine biofilms from surface water. Hence, at this examining

point, the stream water was not polluted with molded waste water from the chose civil sewage (Figure 1).



**Fig. 1: Indication of the deferent biofilm sampling points (BSP)**

### Sampling of biofilms

In light of the experience picked up from past exploration ventures on drinking water frameworks, an altered Robbins gadget method was utilized to test biofilms[9, 10]. Hardened steel fasteners screwed into an empty treated steel chamber (260 mm long, 150 mm in breadth) were held set up by up to 15 tempered steel platelets (450 mm<sup>2</sup>). Punctured plates gave a uniform water appropriation of £ow directly behind the delta and upstream of the outlet of the gadget. Notwithstanding the depicted gadget procedure, for bio-film arrangement, treated steel platelets (2890 mm<sup>2</sup>) were additionally straightforwardly drenched for one month in squander water and surface water. Hardened steel platelets were utilized as the substrate for bio-film development at all examining focuses, while both foundation sides were utilized for the examinations.

### Cultivation of bacteria

Utilizing a sterile scrubber, the bacteria were eliminated from platelet surfaces and pooled in 10 ml of PBS purchaser (10 mM Na-phosphate, pH 7.5, 130 mM NaCl). By the pour plate strategy, for example by plating sequential bacterial suspension weakenings got right this minute scale, the bacteria were quantitated. Kanamycin esculin azide (KAA, Merck Eurolab, and Darmstadt, Germany) for enterococci and Chromocult0 agar (Merck) as a particular specialist for Enterobacteriaceae were utilized in the specific development of bacteria. The bacterial weakenings were put on R2A (Difco) agar (7) for the heterotrophic plate check.

Notwithstanding the protein A-clustering factor latex test (Staphaurex Plus0, Abbott), staphylococci were recognized utilizing the ID32 STAPH pack (BioMerieux, Nu'rtingen, Germany) to recognize *Staphylococcus aureus* and coagulase-negative staphylococci. Enterobacteriaceae disengages were

distinguished by methods for the BioMerieux (API 20E recognizable proof unit). Contrasted and plating tests with no antibiotics in the media, antibiotics were added to the supplement media to assess the level of safe bacteria.

### DNA preparation

Bacteria from 100 ml of fluid media were utilized for DNA confinement as indicated by the maker's directions (DNeasy Tissue Kit, Qiagen, Hilden, Germany) for the arrangement of genomic DNA from refined bacterial strains. Since just a little level of natural bacteria can be developed in engineered media, especially drinking water bacteria [9], genomic DNA was set up from bioelm. Rather than refined planktonic bacteria, bio-lms were scratched onto the platelet surface, suspended in  $10^2$  ml of sterile water, centrifuged for 10 min at 8000Ug, and the pellet was utilized with the above test pack for DNA readiness. The producer's directions were changed to expand the DNA yield. Right off the bat, utilizing the Ribolyser (Hybaid) procedure, mechanical interruption of the bacteria was performed and afterward the lysozyme treatment was stretched out to 1.5 h. As estimated by DAPI (4P,6-diamidin-2P-phenylindole-dihydrochloride, Merck)- recoloring as indicated by Schwartz et al.(8), the bacterial densities of the bio-lms from squander water and surface water were approx.  $10^6$  -  $10^7$  cm<sup>32</sup> bacteria following a multi week hatching period. One significant degree was discovered to be lower in the bacterial densities of drinking water bio-lms, in particular around  $10^5$  cm<sup>32</sup> bacteria. The DNA yields were between 15.0 Wg ml<sup>31</sup> and 180 Wg ml<sup>31</sup> from squander water bio-lms. The measure of DNA arranged from bio-lms of drinking water was more modest (approx.  $2 \times 10$  Wg ml<sup>31</sup> following a month of hatching). Here, separated DNAs were consolidated to raise the centralization of nucleic corrosive.

### PCR and Southern blot hybridization

For PCR, DNA has been utilized from regular bio-lm populaces or refined bacterial strains. Aliquots of suspended bacteria were centrifuged at 5000Ug for 10 min for the screening examination of refined bacteria. The pellets have been resuspended for PCR in 10 Wl of sterile water (Seradest). The GeneAmp0 PCR System 9700 (Applied Biosystems, Weiterstadt, Germany) was utilized to intensify the unique DNA successions. HotStarTaq DNA polymerase (Qiagen) was utilized in the entirety of the PCR tests. At 95 ° C, it was enacted by 15-min hatching. So as to distinguish the antibiotic-opposition genes, diverse temperature profiles were utilized with the particular groundworks (Table 1). The obstruction genes from bio-lms are enhanced by PCR, approx. Genomic DNA of 0.5 Wg was utilized.

As indicated by Uhl et al. (9), PCR frameworks have been utilized to intensify the enterococcal vanA and vanB obstruction genes. As per Klein et al. (10), a

settled vanA genespecific PCR was applied to confirm the primary PCR results. As a positive control, Enterococcus faecium B7641 (vanA; vancomycin least inhibitory focus ( MIC ) s 256 Wg ml<sup>31</sup>; teicoplanin MIC s 16 Wg ml<sup>31</sup>) vancomycin-safe strain was utilized. Utilizing a business extraction unit, genomic DNA from this reference strain was segregated to decide the recognition furthest reaches of this PCR framework. Sequential weakenings were set up from a genomic DNA arrangement of 230-Wg ml<sup>31</sup>. DNA fixations went between 50 pg and 5 fg (in 10 focus steps) in the PCR measures. As far as possible for vanA quality PCR recognition was somewhere in the range of 50 and 500 fg of genomic DNA.

Southern smear hybridization was performed with the digoxigenin-named mecA3-test to distinguish the methicillin-opposition quality in staphylococci (Table 1). Here, the objective arrangement inside the intensified DNA is found. When the formamide focus was changed in accordance with 20% in the hybridization and washing cradles, explicit hybridization signals were gotten.

### Agar dijuson test for sensitivity testing

For affectability testing, the agar dijuson test strategy portrayed in the German standard DIN 58940 was utilized. As a supplement, Mueller-Hinton agar (BioMerieux) is utilized. E. ATCC 29212 and E faecalis. ATCC 6057 faecium filled in as the control strain.

### DNA sequencing and analysis

For sequencing examines, enhanced DNA pieces from PCR tests were utilized. The DNA was removed from an agarose gel and purged by the producer's directions with a QIAquick1 gel extraction unit (Qiagen). GENterprise (Mainz, Germany) performed sequencing, utilizing forward and invert groundworks explicit for obstruction quality intensification. Looks for FASTA and BLAST DNA homology were performed utilizing the NIH (USA) DNA information base software got from the Internet address: <http://www.ncbi.nlm.nih.gov>.

## RESULTS

### Enterococci/streptococci

In medical clinic biofilms, the enterococci/streptococci loads and the presence of safe bacteria were most prominent. Biofilms from enacted muck demonstrated marginally lower province means enterococci/streptococci at the metropolitan sewage treatment plant. At the outlet end of the molded waste water from the office, another decline in the bio-lm load was watched. Just limited quantities of enterococci/streptococci were found in surface water



biofilms, and none of them were biofilms detached from drinking water.

The agar dispersion test decided the opposition examples of 39 vancomycin-safe enterococci. Antibiotic medication and erythromycin were additionally impervious to these bacteria. Ampicillin (62 percent), amoxicillin/clavulanic corrosive (33 percent), imipenem (51 percent) and gentamicin (59 percent) were recorded with significant levels of opposition. Protection from cipro-£oxacin (2.5%) and cotrimoxazole (12.8%) was low, then again.

**Enterobacteriaceae**

The most noteworthy heap of Enterobacteriaceae in medical clinic bioelms was watched, like the outcomes got for enterococci/streptococci, trailed by bioelms from actuated muck of the metropolitan wastewater therapy plant (Table 1). At the initiated muck testing point, the bacterial cell check at the plant's outlet was 72 percent lower than the cell tally. Another decrease in the heap of Enterobacteriaceae was estimated in bio-lms of surface water and no Enterobacteriaceae were identified in bio-lms of drinking water. In bio-lms from medical clinic wastewater (54 O 15 percent), the biggest number of cefazolin-safe Enterobacteriaceae was estimated, trailed by initiated slime bio-lms (19 O 12 percent) and plant release with 11 O 4.1 percent. 27 O 10% was the rate opposition in surface water bio-lms. In medical clinic wastewater bio-lms, the most elevated level of cefotaxime obstruction was discovered (17 O 7.5 percent), trailed by actuated ooze bio-lms (5.3 O 0.6 percent) and plant release (1.9 O 0.4 percent). No Enterobacteriaceae impervious to cefotaxime were secluded from bio-lms of surface water.

A serious extent of homology (71 percent by BLAST) was found among Enterobacter cloacae (GenBank AB016611), Escherichia coli (GenBank AF124205), and Citrobacter freundii (GenBank X76636) concerning the ampC obstruction quality. Inside the agreement districts of the coding succession of ampC, groundworks were resolved. Table 1 presents the recently planned PCR groundworks ampC-For and ampC-Rev.

**Table 1: Bacteria from bioelms cultivated on dijferent media**

	Hospital (n=30)	Activated sludge at municipal sewage (n=3)	Efflu of municipal sewage (n=3)	Surface water (n=3-8)	Drinking water (n=6)
Enterococci and streptococci on SCAA medium CFU (n <sup>-1</sup> without antibiotics)	46 (1.36)	43.3 (2.2)	26 (3.6)	4	0
Enterobacteriaceae on Chromocult medium CFU (n <sup>-1</sup> without antibiotics)	8.7 x 10 <sup>4</sup> (2.34 x 10 <sup>5</sup> )	1.6 x 10 <sup>4</sup> (2.65 x 10 <sup>4</sup> )	445 (2.19)	81 (2.2)	0
Heterotrophic bacteria on R2A medium CFU (n <sup>-1</sup> without antibiotics)	5.8 x 10 <sup>4</sup> (1.37 x 10 <sup>5</sup> )	3.8 x 10 <sup>4</sup> (1.32 x 10 <sup>5</sup> )	1.2 x 10 <sup>4</sup> (3.88 x 10 <sup>4</sup> )	8 x 10 <sup>3</sup> (1.1 x 10 <sup>4</sup> )	1.8 x 10 <sup>3</sup> (1.75 x 10 <sup>3</sup> )

**Staphylococci Genomic**

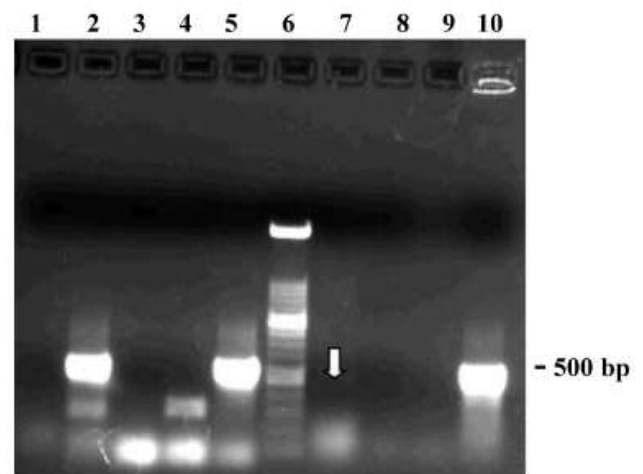
For PCR recognition of the mecA quality, DNA from bio-lms was utilized (Table 1). Explicit amplicons were

just identified from clinic wastewater in the bio-lm; as far as the mecA obstruction quality, the other examining focuses were negative (Table 2). Just one of three examples created a powerless sign in clinic bio-lms after the main PCR. A more grounded signal was found in a second PCR examine with 2-WI aliquots of the principal PCR arrangement (Fig . 2). With the marked mecA3 test, the agarose gel was smudged and hybridized. The hybridization explore denounced the presence of low staphylococcal mecA obstruction quality focuses in emergency clinic squander water bio-lms. Additionally, in a twofold abandoned way, the PCR amplicon was sequenced. The mecA quality was perceived in S by a BLAST nucleotide search. With aureus, S. Epidermidis, just as S. Sciuri (96 percent each).

To assess the determination of staphylococci in emergency clinic bio-lms, equal development tests were directed. Following manufactured medium enhancement [20], S. With aureus, S. Chromogenes, just as S. They developed epidermidis. Of the apparent multitude of separated staphylococci, just the S. The epidermidis disengage was methicillin safe. These outcomes were affirmed by PCR and hybridization tests.

**Table 2: Detection of resistance genes in bacteria and DNA prepared from bioelms**

Biofilm sampling point	Cultivated resistant bacteria	Total DNA from biofilms
Vancomycin resistance for enterococci; detected resistance gene: vanA		
Wastewater systems		
• Hospital (n=4)	positive	positive
• Municipal sewage (n=8)	positive	positive
• River water (n=4)	negative	positive
• Drinking water (n=8)	negative	positive
Methicillin resistance for staphylococci; detected resistance gene: mecA		
Wastewater systems		
• Hospital (n=4)	positive	positive
• Municipal sewage (n=8)	not tested	negative
• River water (n=4)	not tested	negative
• Drinking water (n=8)	negative	negative
β-Lactams resistance for Enterobacteriaceae; detected resistance gene: ampC		
Wastewater systems		
• Hospital (n=3)	not tested	positive
• Municipal sewage (n=8)	positive	positive
• River water (n=4)	positive	positive
• Drinking water (n=3)	negative	positive



**Figure 2: Detection of the mecA gene coding for methicillin resistance in staphylococci**

**Heterotrophic bacteria**

So as to get data about culturable bacteria in bio-lms and to explore whether vanA and ampC opposition

genes were available in culturable or non-culturable drinking water bacteria, heterotrophic bacteria were broke down and portrayed. In emergency clinic bio-lms, the heterotrophic bacteria tally was most elevated (Table 1). In surface and drinking water bio-lms, lower cell checks were found. Because of the structure of the R2A medium, which is normally utilized for the list and subculture of oligotrophic bacteria from drinking water, heterotrophic plate includes in clinic wastewater frameworks were discovered to be lower than enterobacterial state tallies (7), however victimized the development of Enterobacteriaceae. All bio-lms, including drinking water bio-lms, were developed from heterotrophic bacteria impervious to vancomycin, ceftazidime, cefazolin and penicillin G (Table 3). Imipenem-safe heterotrophic bacteria have not been created from bio-lms of drinking water. PCR explores different avenues regarding drinking water bacteria were acted so as to choose whether any of these obstruction systems depended on the presence of the vanA quality for vancomycin or the ampC quality for L-lactam. For this reason, the safe bacteria were pooled, and genomic DNA was removed. VanA and ampC-speci'c PCR didn't create separate amplicons. Moreover, bio-lms heterotrophic drinking water bacteria that were filled without antibiotics demonstrated no vanA or ampC-speci'c amplicons, showing that there were no cultivable bacteria with vanA or ampC obstruction genes in bio-lms drinking water. Moreover, in the presence or nonattendance of antibiotics by PCR and hybridization tries, no enterococcal-speci 23S rDNA-related arrangements were distinguished in developed drinking water bacteria from bio-lms.

**Table 3: Percentage of antibiotic-resistant heterotrophic bacteria in bioϕlms**

	Hospital (n=5)	Artificial storage at artificial storage (n=5)	Office of hospital storage (n=5)	Surface water (n=5)	Drinking water (n=5)
Vancomycin <sup>r</sup>	80 ± 1.61	70 ± 1.41	110 ± 1.10	50 ± 0.50	30 ± 0.30
Ceftazidime <sup>r</sup>	80 ± 1.61	90 ± 0.91	275 ± 1.15	100 ± 1.00	5 ± 0.20
Cefazolin <sup>r</sup>	80 ± 1.61	70 ± 0.71	30 ± 0.30	8.00	40 ± 2.71
Penicillin G <sup>r</sup>	70 ± 1.20	30 ± 0.30	20 ± 0.20	30 ± 1.51	40 ± 2.01
Imipenem <sup>r</sup>	80 ± 1.61	100 ± 0.01	110 ± 0.01	80 ± 0.01	0

## DISCUSSION AND CONCLUSION

The inception of the obstruction quality arrangements must be addressed considering these changes. It is realized that in microorganisms other than enterococci, for example, *Paenibacillus popilliae*, *Amycolatopsis orientalis*, and *Streptomyces toyocaensi* (11), quality groups homologous to vanA and vanB exist. Heterotrophic bacteria were subsequently developed and tried for the event of the opposition genes vanA and ampC from drinking water bio-lms. No such genes have been distinguished, and it must be inferred that these obstruction genes are absent in these microorganisms that can be developed. In any case, the chance cannot be precluded that connected obstruction genes of lower homology grant protection from these bacteria and that the enterococcal and enterobacterial-speci-c groundworks utilized for vanA and ampC cannot distinguish these opposition genes.

In concentrated consideration units, another critical pathogenic operator is *S. aureus* (14). In methicillin-safe S diseases. Because of protection from all other L-lactam antibiotics, aureus strains are accessible with a couple antibiotics, for example, vancomycin or teicoplanin (12). Since 1990, protection from methicillin by *S. aureus*. Because of the restricted helpful prospects it suggests, aureus has expanded significantly (13) and is of specific concern. Staphylococci were just found in clinic squander water bio-lms in this study. The quality *mecA* was identified, however just methicillin-safe coagulase-negative strains and not methicillin-safe *S. aureus* were found in development tests. Strains of aureus.

From the outcomes, it is inferred that Vancomycin-safe enterococci and L-lactam-hydrolysing Enterobacteriaceae were developed from all bio-lms of waste water and were less as often as possible found in surface water bio-lms. Enterococci's vanA vancomycin-obstruction quality, staphylococci's *mecA* methicillin-opposition quality, and Enterobacteriaceae's ampC L-lactam-opposition quality were extended basically from clinic wastewater bio-lms. In all other waste water and ecological bio-lms, VanA genes and ampC genes were likewise distinguished. In drinking water bio-lms, the vanA and ampC antibiotic-obstruction genes were recognized, while enterococci and enterobacteriaceae, initially said to convey these genes, were never found in these bio-lms. The enhanced genes give off an impression of being important for the genome of practical yet non-cultivable amphibian bacteria.

## REFERENCES

1. WHO & WTO. (2002) WTO Agreements and Public Health: A Joint Study by The WHO and The WTO Secretariat; World Health Organization, World Trade Organization Secretariat: Geneva, Switzerland.
2. Emmerson, A.; Jones, A. (2003) The quinolones: Decades of development and use. *J. Antimicrob. Chemother.* 51, pp. 13–20.
3. Hawkey, P. M., and Jones, A. M. (2009). The changing epidemiology of resistance. *J. Antimicrob. Chemother.* 64(Suppl. 1), i3–i10. doi: 10.1093/jac/dkp256
4. Bahl, M. I., Sorensen, S. J., Hansen, L. H., and Licht, T. R. (2004). Effect of tetracycline on transfer and establishment of the tetracycline-inducible conjugative transposon Tn916 in the guts of gnotobiotic rats. *Appl. Environ. Microbiol.* 70, pp. 758–764. doi: 10.1128/AEM.70.2.758-764.

5. Cole, D., Drum, D. J., Stalknecht, D. E., White, D. G., Lee, M. D., Ayers, S., et al. (2005). Free-living Canada geese and antimicrobial resistance. *Emerg. Infect. Dis.* 11, pp. 935–938. doi: 10.3201/eid1106.040717
6. Lee YJ, Han HS, Seong CN, Lee HY, Jung JS (1998) Distribution of genes coding for aminoglycoside acetyltransferases in gentamicin resistant bacteria isolated from aquatic environment. *J Microbiol* 36: pp. 249–255
7. Reasoner, D.J. and Geldreich, E. (1985) A new medium for enumeration and subculture of bacteria from potable water. *Appl. Environ. Microbiol.* 49, pp. 1-7.
8. Schwartz, T., Hojmann, S. and Obst, U. (1998) Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. *Water Res.* 32, pp. 2787-2797.
9. Uhl, J., Kohner, P., Hopkins, M. and Cockerill, F.R. (1997) Multiplex PCR detection of vanA, vanB, vanC-1, and vanC-2/3 genes in enterococci. *J. Clin. Microbiol.* 35, pp. 703-707.
10. Klein, G., Pack, A. and Reuter, G. (1998) Antibiotic resistance pattern of enterococci and occurrence of vancomycin-resistant enterococci in raw minced beef and pork in Germany. *Appl. Environ. Microbiol.* 64, pp. 1825-1830.
11. Patel, R. (2000) Enterococcal glycopeptide resistance genes in nonenterococcal organisms. *FEMS Microbiol. Lett.* 185, pp. 1-7.
12. Hryniewicz, W. (1999) Epidemiology of MRSA. *Infection* 27, pp. 13-16.
13. Kresken, M., Hafner, D. and Rosenstiel, N. (1999) Development of antibiotic resistance in clinically relevant microorganisms in central Europe. *Bundesgesundheitsbl.-Gesundheitsforsch.-Gesundheitsschutz* 42, pp. 17-25.
14. Vercauteren, E., Descheemaeker, P., Ieven, M., Sanders, C. and Goossens, H. (1997) Comparison of screening methods for detection of extended-spectrum L-lactamases and their prevalence among blood isolates of *Escherichia coli* and *Klebsiella* spp. in a Belgian teaching hospital. *J. Clin. Microbiol.* 35, pp. 2191-2197.

---

### Corresponding Author

**Dr. Nitin Kumar\***

Assistant Professor, Department of Chemistry, Jai Murat Rai Degree College, Nirpur, Patepur, Vaishali