

# IWRA's XVII WORLD WATER CONGRESS

제 17차 IWRA 세계물총회

29 November – 3 December 2021  
EXCO, Daegu, Republic of Korea



# Antibiotic Resistant Genes and Integrons: A potential threat to the Ganga river ecosystem, India



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*Submission no. AO-D2-0596*

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# Introduction



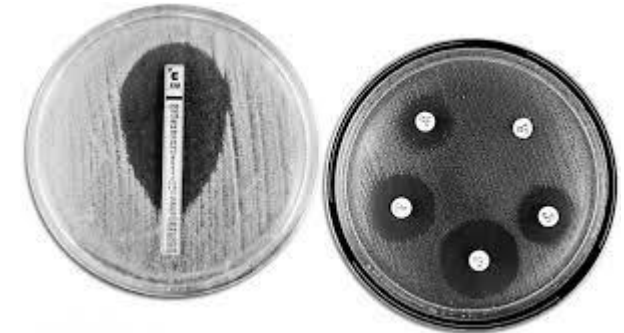
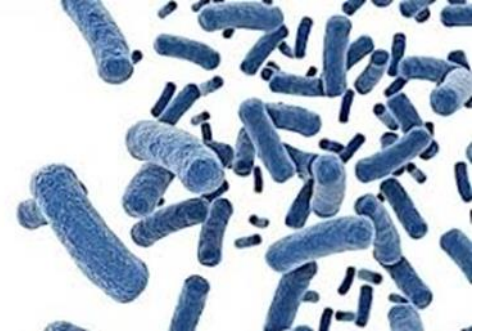
- ❖ Rapid urbanization has led to the degradation of river systems
- ❖ Economic/sociocultural/ecological services - supports a large population in sustaining diverse life forms
- ❖ Water & microbial pollution are cause of concern

*River pollution - **significant threat to public health** - due to microbes - various exposure pathways - dissemination of ARG/ARB*



## ***SO WHAT IS ANTIBIOTIC RESISTANCE?***

- ❖ Exposure of pathogenic bacteria and *E.coli* to antibiotics - acquisition of multidrug resistance in the bacterial population
- ❖ Antimicrobial resistance affects the aquatic species, and also various stakeholder's dependent for different purposes



# What does World Health Organization has to say about AMR ??



AMR is a global health and development threat. It requires urgent multisectoral action in order to achieve the Sustainable Development Goals (SDGs)

WHO has declared AMR as one of the top 10 global public health threats facing humanity

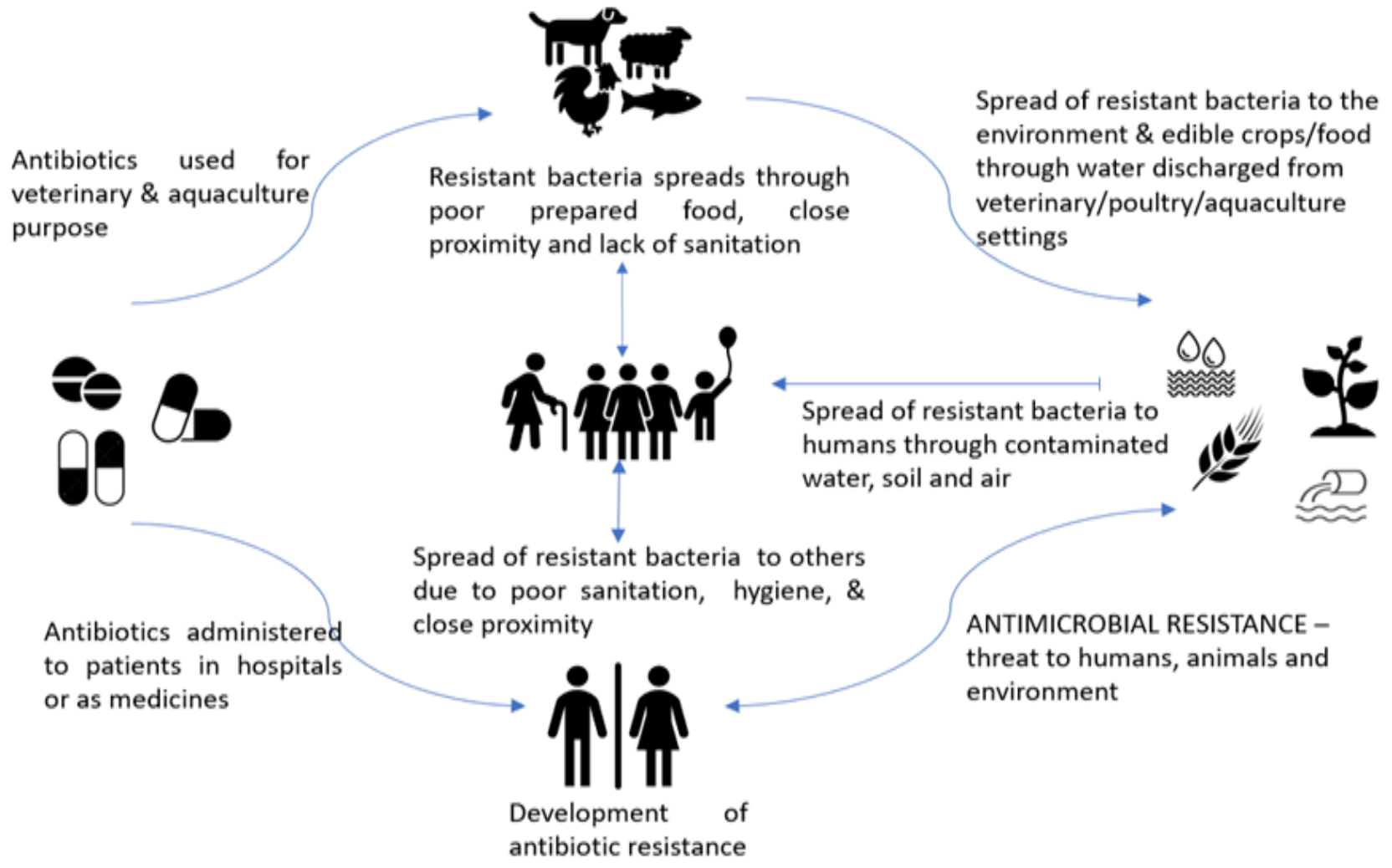
Misuse/overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens

Lack of clean water/sanitation/inadequate infection prevention/control promotes the spread of microbes, some of which can be resistant to antimicrobial treatment

The cost of AMR to the economy is significant. In addition to death and disability, prolonged illness results in longer hospital stays, the need for more expensive medicines/financial challenges for those impacted

Without effective antimicrobials, the success of modern medicine in treating infections, including during major surgery and cancer chemotherapy, would be at increased risk

# Significant threat and pathways of antibiotic resistance to aquatic environment and human health



# Characteristics and potential threats of ARGs



<b>Types of ARG/MGE</b>	<b>Characteristics</b>	<b>Health threat</b>
NDM	<ul style="list-style-type: none"> <li>❖ Ubiquitous in DNA materials, including mobile genetic elements (MGEs, e.g., plasmids and transposons) (Partridge et al., 2009)</li> <li>❖ Constitutes about 9% of sequenced bacterial genomes</li> </ul>	<ul style="list-style-type: none"> <li>❖ Cause of concern because they encode for <i>E.coli</i> leading to urinary tract infection that leads to hospitalization and transfer between hospital sectors to communities.</li> </ul>
Integron1	<ul style="list-style-type: none"> <li>❖ Indicator of fecal pollution and biomarker of direct anthropogenic impact on river systems (Ma et al. 2017)</li> <li>❖ Indicates release of effluent containing ARB (Stalder et al., 2014)</li> <li>❖ Significant role for dissemination of antibiotic resistance</li> <li>❖ Not efficiently removed by WWT &amp; abundance increases downstream of the treatment plant</li> <li>❖ Diversity of integrons in polluted water indicates their role in adaptation of bacterial communities in response to major environmental disturbances (Abella et al., 2015)</li> </ul>	<ul style="list-style-type: none"> <li>❖ Associated with pesticide pollution</li> <li>❖ <i>int1</i> released through sludge disposal &amp; effluents of treatment plant directly impacting riverine system</li> <li>❖ High concentration detected in surface water (Li et al., 2010)</li> <li>❖ <i>Enterobacter cloacea</i> with <i>int1</i> have been isolated from prawns and biofilm of drinking water supplies (Gillings et al., 2009)</li> </ul>



<b><i>Types of ARG/MGE</i></b>	<b><i>Characteristics</i></b>	<b><i>Health threat</i></b>
Integron2	<ul style="list-style-type: none"><li>❖ Associated to cattle &amp; wild animals (Chainier et al., 2017)</li><li>❖ Abundant in diverse range of pathogens, commensals, &amp; environmental microorganisms</li><li>❖ Capable of replication so possess properties of both pollutants and invasive species (Radstrom et al., 1994)</li></ul>	<ul style="list-style-type: none"><li>❖ Use of animal manures impacts soil ecology</li></ul>
Integron3	<ul style="list-style-type: none"><li>❖ Evolutionary history similar to that of integron1</li><li>❖ Continuously colonizing new bacterial species, acquiring novel resistance cassettes, &amp; making its way onto new plasmid vectors (Gillings, 2014)</li></ul>	<ul style="list-style-type: none"><li>❖ Detected in effluents of treatment plants &amp; slaughter house (Li et al., 2010)</li><li>❖ Isolated from sludge (Xu et al., 2007), alters the microbial ecology of agricultural land after application of sludge as fertilizer</li><li>❖ Isolated from oysters (Barkovskii et al., 2010), enters the food chain to transit between environment &amp; humans</li></ul>



# Objective of the present study

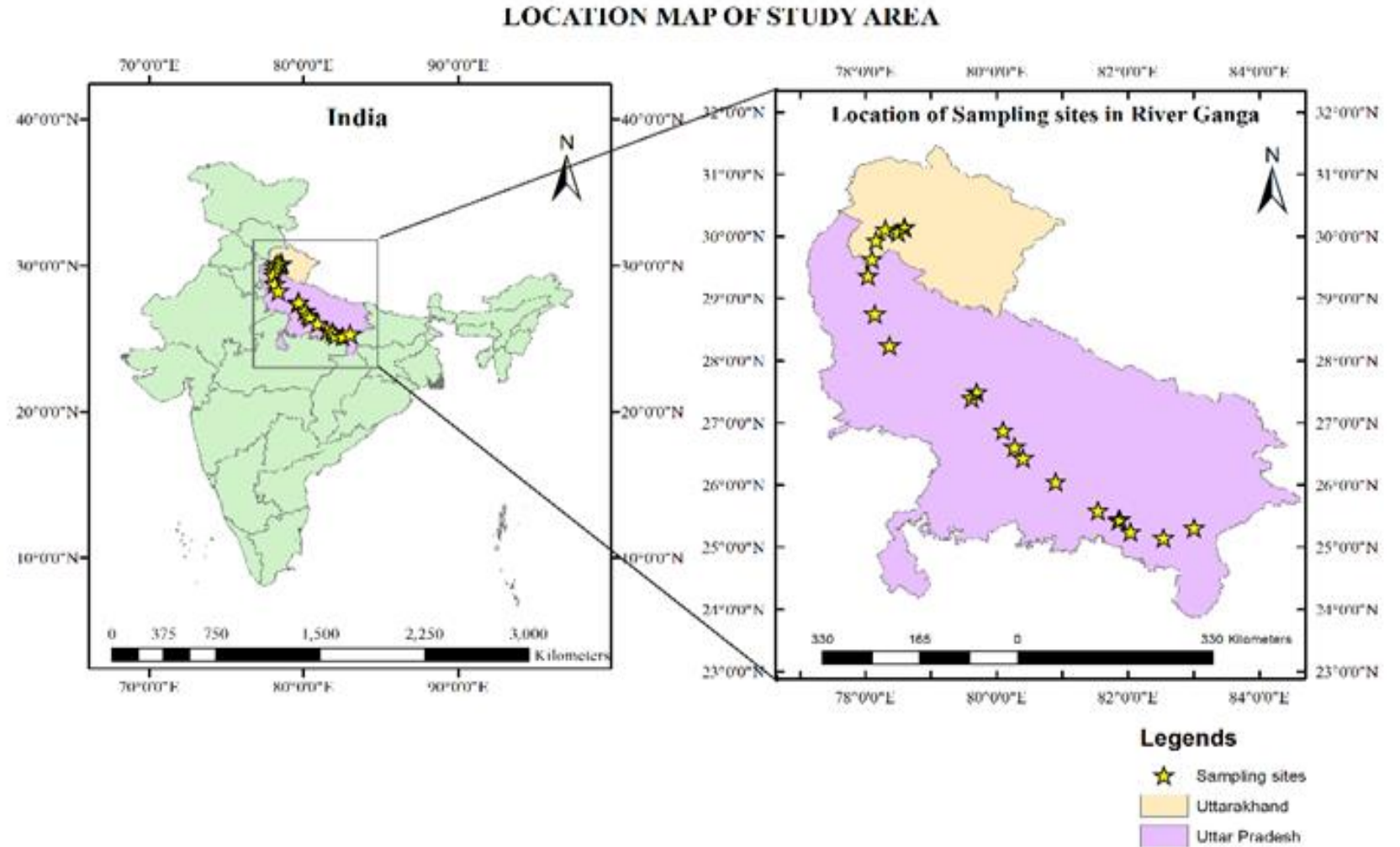


- ❖ Explores the association between ARGs (especially NDM) with integrons, and their unique pattern along the entire upper and middle plain region of river Ganga
- ❖ Need of strengthening global surveillance of AR in the important riverine systems with a stronger focus on adopting stringent measures for managing microbial contaminants to avoid health threats

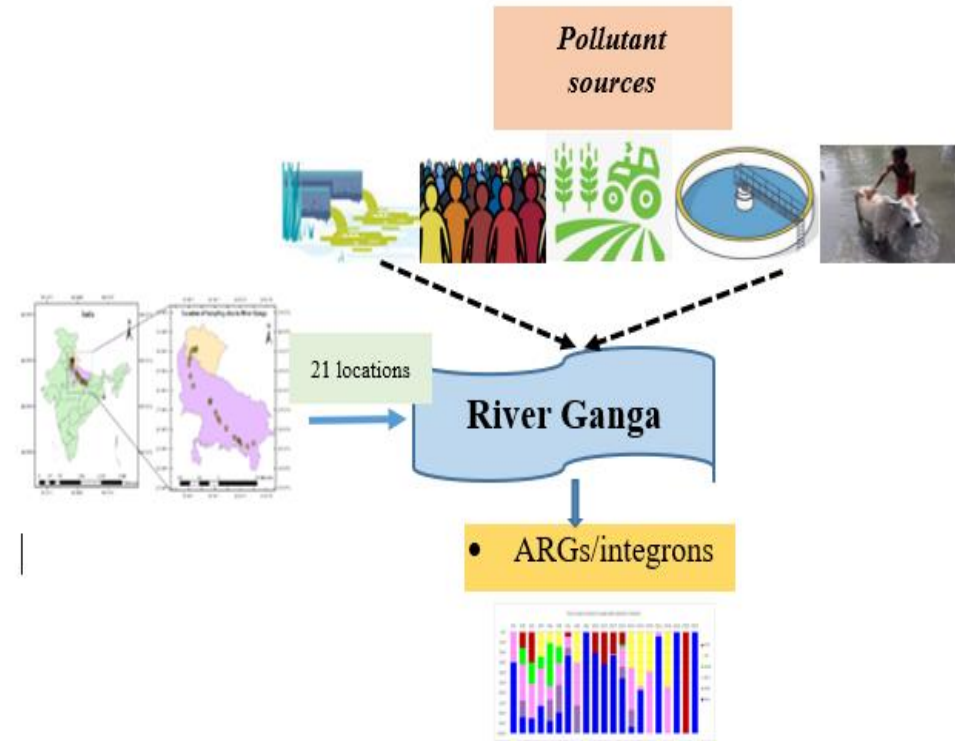
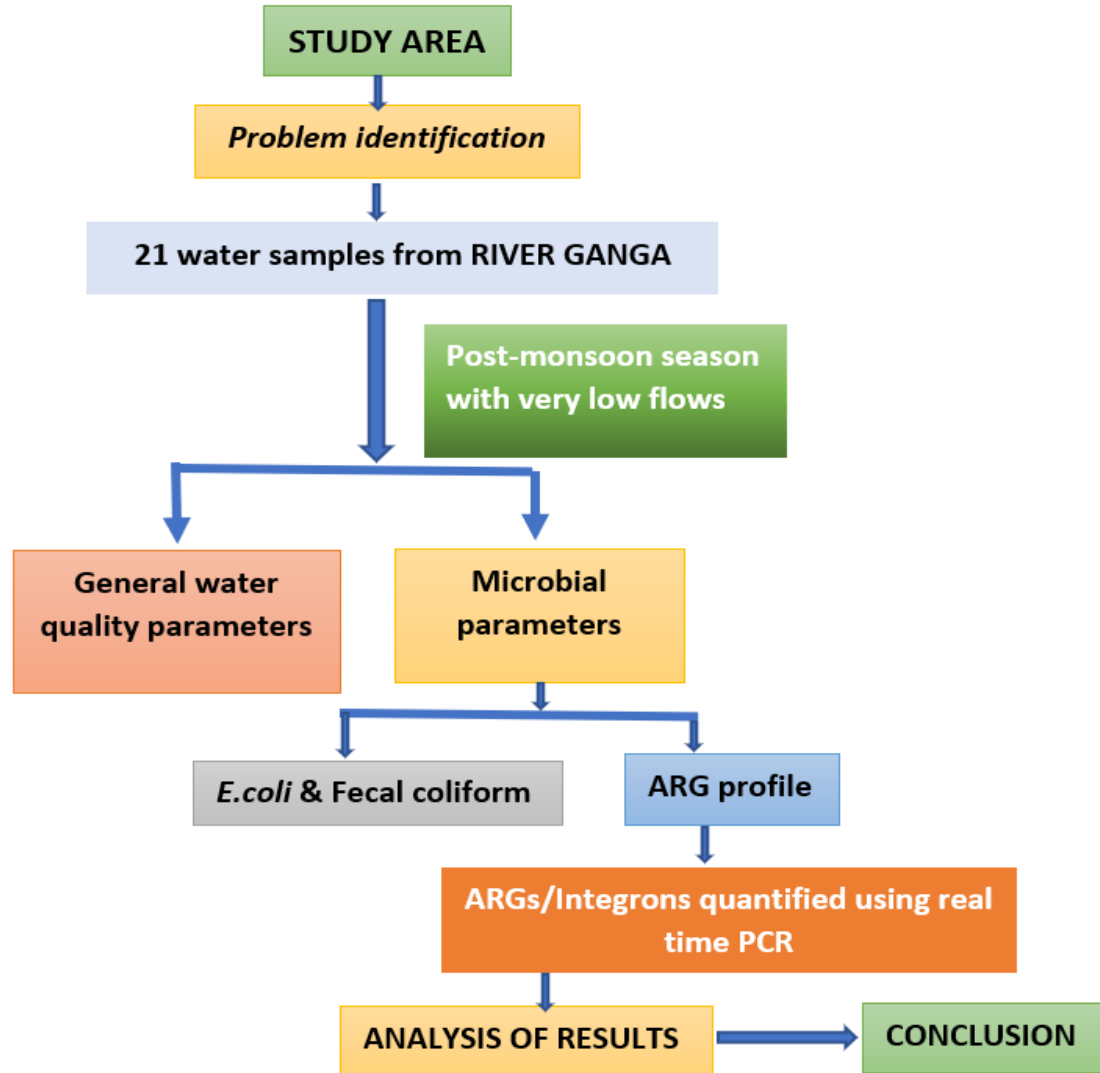
# Study area



**Figure 1:** 21 locations sampled along the upper and middle stretch of river Ganga

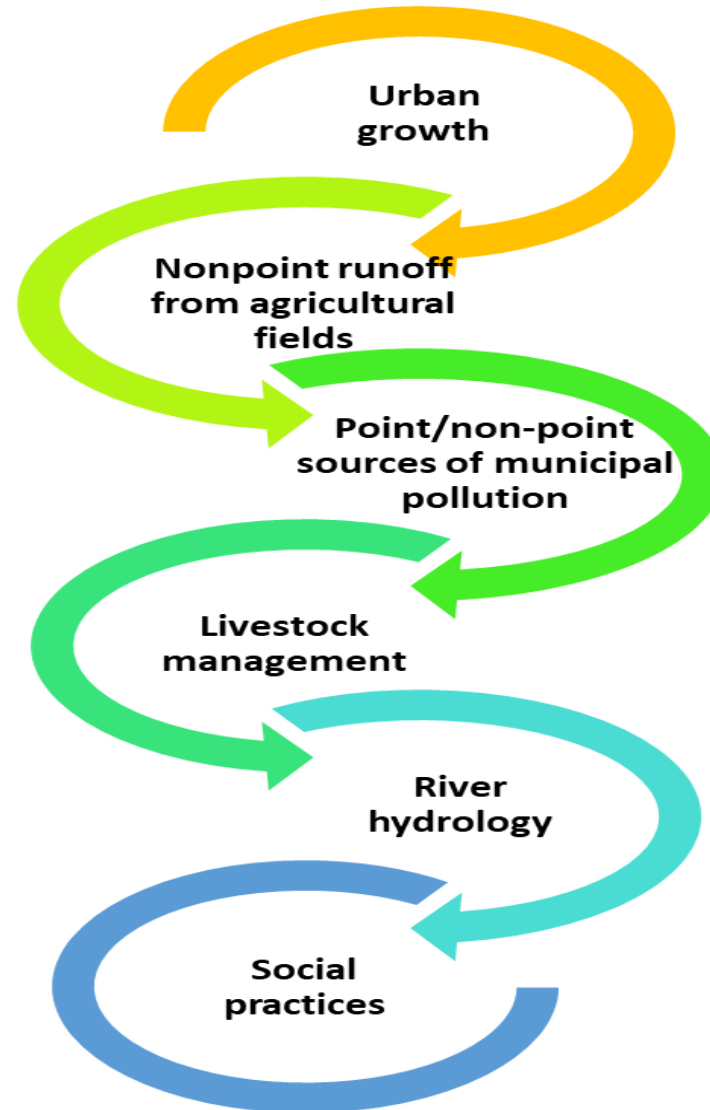


# Methodology

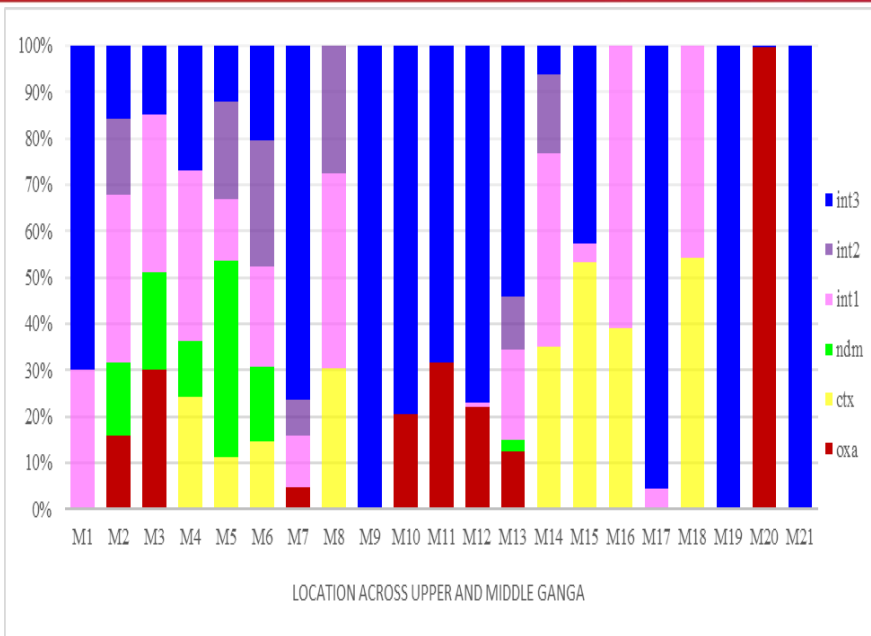


**Figure 2a & 2b:** Flow diagram and graphical representation of the present study

# Risk factors in river Ganga basin



# Occurrence and diversity of antibiotic resistant genes and integrons



**Figure3: Association of resistant genes along the upper and middle stretch of River Ganga**

- ❖ Highest number of ARGs/integrons - upper stretch - to a maximum of 5 genes
- ❖ NDM – unique signature - abundant - upper stretch - varied between 11.86-42.27%
- ❖ M13 – NDM – in very low % (2.56%) with respect to total relative abundance – no other location with NDM reported in the middle stretch
- ❖ OXA & CTX – ranges from 12.43 – 99.74% & 11.26 – 54.19% - *max reported in middle stretch*
- ❖ Dominance of *int 3* in the range of about 6.27-100%, over *int 1* and *int 2*
- ❖ 3 locations – M8, M16 & M18 - *int 3* absent
- ❖ M8 shows the dominance of *int 1* & *int 2* (~70% in total), likewise M16 & M18 only *int 1* (45-62%)
- ❖ M1 indicated highest percentage of *int 3* along with *int 1* – with no other genes
- ❖ M9, M19, M21 - 100% of *int 3* – tributaries/anthropogenic interferences add to the pollutant load - the percentage reaches to maximum due to its wide spread in the riverine system.

## Some eminent research:

- ✓ Chaturvedi et al. (2021) – *int* in river Ganga, contributor for spread of resistance
- ✓ Toleman et al. (2015) – NDM migrates from hospital wastes to environmental components (soil & water)
- ✓ Ahammad et al. (2014) – upper stretch of river Ganga – abundance of NDM – due to holy rituals & visits
- ✓ Kaushik et al. (2019) – ARG/int isolated from Indian rivers
- ✓ Singh & Verdi (2017) – ARG/int – *E.coli* - isolated from river Yamuna – transmitters of resistant genes

# Key findings

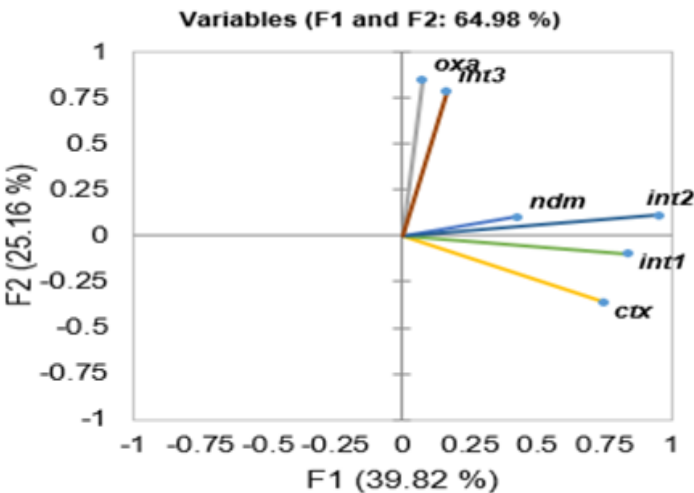
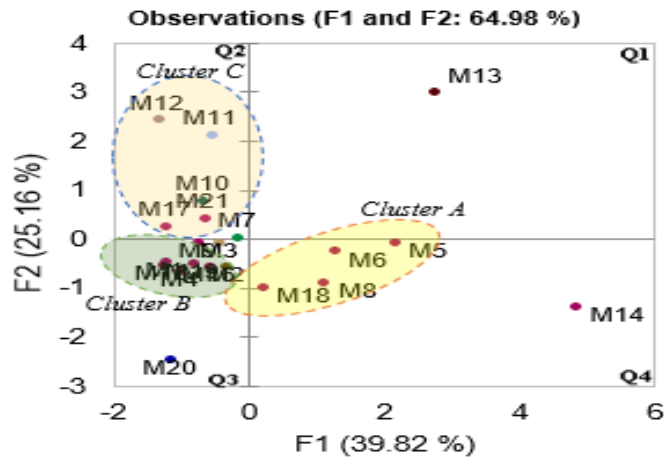


Fig 4a: Clusters grouping different locations

Fig 4b: PcoA analysis demonstrating the association of ARGs with integrons

- ❖ 3 clusters based on their similarity with respect to abundance and diversity of ARGs
- ❖ PCoA based on the Bray–Curtis distance of relative abundance of ARGs showed that few locations were distinct from the others (M13, 14 and 20) - indicate maximum number of total ARG/int or OXA being dominant
- ❖ PCA analysis clearly differentiates oxa and *int 3* from rest of the integrons and resistant genes that lie quite apart from them
- ❖ Association of NDM with int
- ❖ Positive correlation - between ctx with *int 1* and *int 2*
- ❖ Results indicate *int 3* for dissemination of oxacillinase gene in environmental sample and also the association of ctx with *int 1* and NDM with *int 2*
- ❑ *Dominance of integrons – does not allow the abundance of other genes in higher %*
- ❑ *Non-point sources also contribute to the dominance of integrons*

# Conclusion



- ❖ Indicates the abundance of ARG and integrons in the Ganga River during the critical low flow period
- ❖ River - reservoir of resistant genes - worsened due to joining of the tributaries
- ❖ Potential risk - resistant genes/integrons on the aquatic environment as well as human health
- ❖ Public awareness & surveillance
- ❖ Further research needed to assess the possible influence of different seasons and the abundance, association of these resistant genes with other water quality parameters,
- ❖ Mechanisms of transfer and fate of ARG in the riverine ecosystem



# References and further readings



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## WHO GUIDELINES FOR ANTIBIOTIC RESISTANCE

- <https://www.who.int/health-topics/antimicrobial-resistance>
- <https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance>

<https://www.cdc.gov/drugresistance/index.html>



*Thank you....*



*If there is magic on this planet, it is contained in water*



***Specialization:***

- Wastewater treatment (wrt water and microbial pollutants)
- Phytoremediation & reclamation technologies for decentralized systems (natural/CWs) - Ramsar site (Renuka Wetland in Northern India)
- Water accounting
- Microbial contaminants in the effluent discharges from WWTP

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