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Practical Notes for Quantification of Antibiotics Resistant Bacteria and Antibiotics Resistance Genes in Wastewater

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Abstract: The occurrence of new chemical and microbiological contaminants in the aquatic environment has become an issue of increasing environmental concern. Integrated antibiotic resistance surveillance is one of the objectives of the World Health Organization global action plan on antimicrobial resistance. Urban wastewater treatment plants (UWWTPs) are among the most important receptors and as a spot of antibiotic – Bacteria interaction. Thus, wastewater treatment plants (WWTPs) play an important part in the dissemination of so-called new emerging pathogens and antibiotic resistances. So, the periodical survey of antibiotic resistance genes in WWTP and finding new techniques consider the best step to reducing this emergence problem.

Key words; Antibiotics resistance, Wastewater, resistance bacteria

1.Introduction

Waste water treatment plants (WWTPs) and Wastewater (WW) play as big reservoirs for both antibiotic resistance bacteria (ARB) and antibiotics residues (AR). and due to incomplete metabolism of antibiotics in humans or by disposal of unused antibiotics this WWTPs will be a disseminating spots for ARB & AR (Bouki C et al. 2013). Since Waste water contains a complex combination of different chemical material like disinfectants, antibiotics and heavy metals which can trigger antibiotics resistance by selection pressure, even in low concentrations. Also can increase the chance of antibiotics resistance because WWTP consider interaction area for Antibiotics and Bacteria from Different Sources (Karkman A et al . 2018). Every day municipality WWTPs receive wastes that full of bacteria and antimicrobial agents from various sources, making it ideal environment microbes to interact and exchange genes horizontally with different microbial species (Berendonk T.U et al. 2015; Martínez J.L. 2009; Pal C et al. 2016)

Recent reports estimates that, by 2050, 10 million people will die every year due to antibiotics resistance unless a global societies response to this problem (Kraker M.E.A et al. 2016). Because of this problem expanding day after day (Shadman T.S &Yasa I. 2018). it must be there periodical research and development for assessment of ARB & ARG from waste water and apply new practical methods to enhance the quantification and identification of these pollutants.

Therefore, according to our experienceses in this field we highlights some practical nots to be taked in considerations by other researchers whene they starting this kinde researches.

2.ARB & ARGs quantification methods

For the past 70 years, research in antibiotic resistance has focused mainly on pathogens. Isolating microbial pure cultures still the most important method in microbiology. In the other side antibiotic susceptibility testing of bacteria is relatively inexpensive and gives important data on resistance patterns that are needed for the clinical treatment of patients (Karkman A et al. 2018). Most scientific reports showed that ARGs identification studies have focused on the use of real-time PCR (polymerase chain reaction) or real-time quantitative PCR (Q-PCR).

Now days (Q-PCR) amplification is widely applied for determining ARG within environmental samples without

the need for culturing especially in WWTPs (Zhu Y G et al. 2013; Yuan Q et al. 2016; Turolla M et al. 2018).
Culturing based methods give us the number of total bacterial cells but cannot give us real quantity of resistance bacteria and ARG, for this reason the best method is using PCR technique.

In the following we will put some notes in researchers hand to be followed when start to work on ARB & ARGs:-

1- Study of UWWTP Samples separately from industrial or hospital WWTPs.
2- Starting with the most commonly used antibiotics in specific geographic of sampling site to make a survey about the amount of antibiotics eliminated to the environment.
3- Some antibiotics used profusely in winter like erythromycin and some used plenty in other seasons, so must be taken in consideration, this mean that ARG & ARB can be changed according seasons. for examples ARG in *salmonella typhi* and *Vibrio cholera* bacteria can be rare in winter.
4- Collecting water samples in dark glass bottles are better than plastic bottles to avoid any degradations by light or interactions with plastics.
5- Do not collect samples in rainy weather to avoid interacting with water coming from other source out of WWTP canals.
6- Transport samples by ice containers and process within 2- 4 hour.
7- Water samples must be concentrated firstly by filtration through nitrocellulose membranes filters (pore size 0.45 mm) and the filters were rinsed with saline to provide optimium filteration.
8- Using R2A agar medium is better than Luria-Bertani Medium (LB) medium for total bacterial counting and must be immediatly performed after filtration, Max 6h.
9- Filtrate can be store in (- 80 ºC) for DNA extractions in coming days.
10- Antibiotics resistance increased with presence of antibiotics residues,pesticides and heavy metals. So, antibiotics resistance genes survey also need analyses of these materials.
11- Some antibiotics are sensitive for degradation and unstable, like ampicilline and tetracycline, so its recommended to take precautions in the time of sample processe.

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