

*Review*

# Antibiotics and antibiotic resistant bacteria in wastewater: Impact on environment, soil microbial activity and human health

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In recent years, pharmaceutical compounds (PhCs) have aggravated increasing anxiety, particularly as no legitimate requirements have been set for discharge into surface water bodies of these ubiquitous, persistent and biologically active substances. Massive quantities of antibiotics are used in human and veterinary medicines in all parts of the globe to treat diseases with bacterial origins. After administration, antibiotics are excreted by the patient and transmitted in due course to the aquatic environment. These are also largely used in animal operations for growth promotion and for disease prophylaxis. These are often partially metabolized after administration and a significant fraction of the antibiotic can be excreted as the parent compound or in conjugated forms that can be converted back to the parent antibiotic. The residual antibiotics from human and animal use can enter the environment *via* various pathways, including wastewater effluent discharge, runoff from land to which agricultural or human waste has been applied, and leaching which deteriorate the whole ecosystem besides its deleterious impact on human health and aquatic organisms. Antibiotic resistance is a global phenomenon that has severe epidemiological ramifications worldwide and a major peril to public health. This article may give an idea about the sources and fate of commonly used antibiotics detected. More research is needed to quantify the risk of antibiotics in urban wastewater and effluents or surface water so that appropriate action can be taken prior to final discharge into surface water bodies to mitigate the harmful effects on aquatic environment and community health.

**Key words:** Antibiotics, antibiotics resistance, antibiotics resistance bacteria, health hazards, microbial activity, wastewater.

## INTRODUCTION

In the last two decades, occurrence of antibiotics in water bodies and subsequent development of resistance in microorganisms have come into scientific and public focus as an issue of potential concern. Antibiotics can be

defined as organic substances that are produced through the secondary metabolism of living microorganisms or synthesized artificially or semi-artificially that can kill other microorganisms or inhibit their growth or metabolic

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activity via biochemical actions (Lancini and Parenti, 1982; Thomashow and Weller, 1995; Thiele-Bruhn, 2003). Mass production and use of antibiotics in medicine and agriculture have existed and substantially benefited public health and agricultural productivity for over 60 years (Knapp et al., 2010). They have important uses in both human and veterinary medicine for their antibacterial properties and as growth promoters. Their consumption has increased worldwide, and their residues are frequently reported in aquatic environments. It is estimated that the total amounts of annual use of antibiotics had reached 100,000-200,000 tons worldwide (Wang and Tang, 2010), including veterinary antibiotics and medical antibiotics. Among them, veterinary antibiotics are in the majority of the total amount used. For example, they approximately accounted for 70% of the total consumption in the USA and about 70% of them are used for non-therapeutic purposes (Sassman and Lee, 2005; UCS, 2001). China is the largest country in the world for production and use of antibiotics. In 2003, China produced 28,000 and 10,000 tons of penicillin and oxytetracycline (OTC), occupying 60 and 65% of the global total output (Yang et al., 2010), respectively. The total production of major antibiotics in India was more than 2332 million tons in 2006-2007, with a growth rate of 10% (IDMA, 2009).

Medicines consumed for healthcare practices are often released into sewage, and after sewage treatment plant, it reaches the receiving water bodies of lakes or rivers. A huge amount of antibiotics were discharged in water bodies through effluents and get polluted (Dotaniya et al. 2014). Antibiotics used in human medicine are found at trace concentrations in municipal wastewaters, and residual amounts reach ambient waters. Once administered, they are metabolized to varying degrees, and their excreted metabolites and unaltered parent compounds can also undergo further modification due to biological, chemical and physical processes in both sewage treatment facilities and receiving water bodies (Deblonde et al., 2011; Fatta-Kassinos et al., 2011; Miega et al., 2009; Monteiro and Boxall, 2010; Onesios et al., 2009). The majority of the compounds mentioned in the various studies are administered orally, intramuscularly, endovenously or by inhalation, and in few cases on the skin. After administration to humans and animals, up to 90% of the antibiotics can be excreted unchanged via urine and/or feces (Hirsch et al., 1998). These substances are only partially eliminated during sewage treatment (Ternes et al., 2003; Berset et al., 2004). Antibiotics from human sources can enter into the environment through discharge of treated effluents and sludge from domestic wastewater treatment plants (WWTPs) due to incomplete removal, and untreated wastewaters (Batt et al., 2006b; Watkinson et al., 2007; Xu et al., 2007). Therefore, WWTPs play a crucial role in the pollution control of antibiotics to the receiving environments. Antibiotic residues entering in sewage are

directly proportional to their amount used for human and other uses in particular area. The  $\beta$ -lactam antibiotics, which include the penicillins and cephalosporins, represent the largest fraction of human antibiotics, accounting for approximately 18 tones in Switzerland. These were not frequently detected in wastewater because of easy hydrolytic cleavage of the  $\beta$ -lactam ring, despite the fact that  $\beta$ -lactams account for the highest proportion of consumption (Junker et al., 2006).

Antibiotic residues have been detected in different water matrices, including hospital wastewaters (Kummerer, 2001; Lindberg et al., 2004), sewage treatment plant (STP) effluents (Batt et al., 2006; Watkinson et al., 2007; Mutiyar and Mittal, 2013), STP biosolids (Kinney et al., 2006a), soil (Kinney et al., 2006b), surface waters (Kolpin et al., 2002, 2004; Batt et al., 2006), groundwater (Hirsch et al., 1999; Lindsey et al., 2001), sediments (Kerry et al., 1996; Kim and Carlson, 2006), and drinking water (Zuccato et al., 2000, 2005). The first reported case of surface water contamination by antibiotics was in England in 1980, when Watts et al. (1982) detected at least one compound from the macrolide, sulfonamide and tetracycline group of antibiotics in river water at concentrations of  $1 \mu\text{g L}^{-1}$ . Wide range of pharmaceutical formulation is manufactured and used in India, which could lead to the release of more pharmaceuticals substances in the environment (Kurunthachalam, 2012). The PhCs in raw wastewaters are generally in the range of  $10^{-3}$ - $10^{-6}$   $\text{mg L}^{-1}$  (Le Minh et al., 2010; Ziylan and Ince, 2011).

Livestock wastes containing antibiotic residues are directly or indirectly discharged into surface water or applied onto agricultural lands with little treatment. Chlortetracycline is less commonly used in humans, but it is widely used in animals to prevent and treat disease (Kemper, 2008). Similarly, the occurrence of lincomycin has been less reported in domestic wastewater (Watkinson et al., 2007), probably due to its main application in animals (Kemper, 2008). More importantly, significant amount of antibiotics and their bioactive metabolites or degradation products were introduced in agro-ecosystems through fertilization and irrigation with antibiotics-polluted manures, biosolids, sewage sludge, sediments and water. Subsequently, accumulation and transport of antibiotics in soil-crop systems, particularly soil-vegetable systems, for example, protected vegetable and organic vegetable production systems, poses great risks on crops, soil ecosystem and quality of groundwater and plant-based products (Ramana et al. 2002; Lianfeng and Wenke, 2012). These results are reported with a view to aiding scientists and administrators in planning measures aiming to reduce the impact of antibiotics in treated urban wastewater discharge into surface water bodies.

## **MOST COMMONLY USED ANTIBIOTICS**

Antibiotics belonging to different classes ( $\beta$ -lactams,

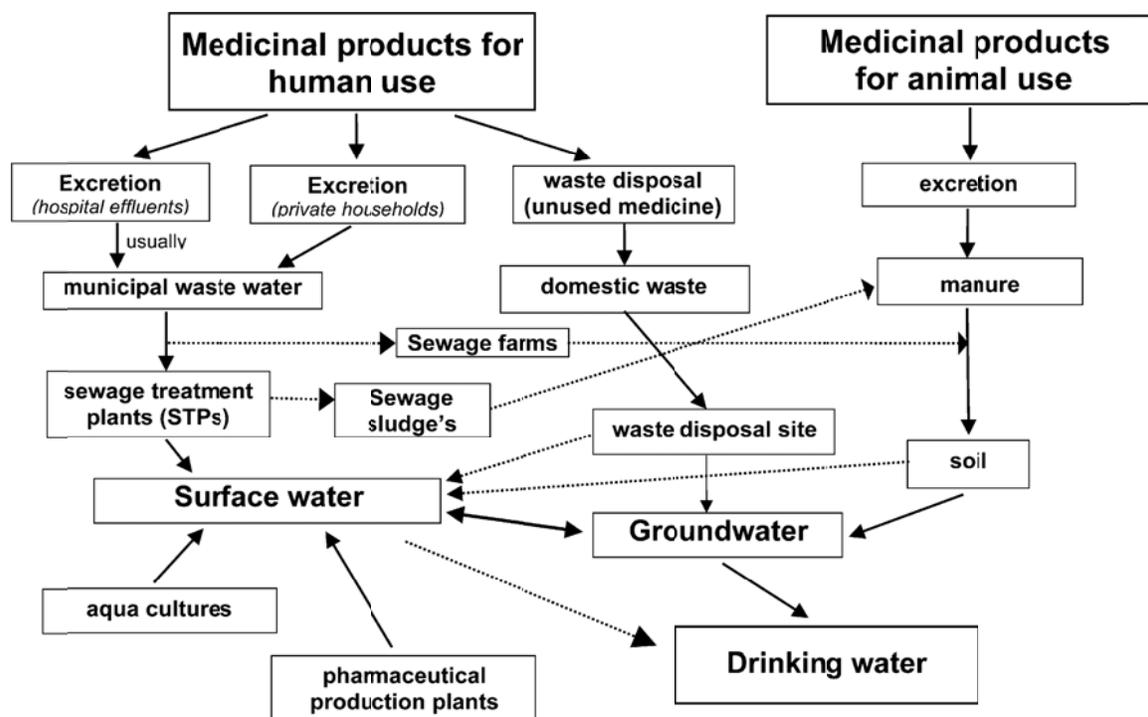
**Table 1.** Most commonly used antibiotics with their class/subclass, molecular weight (MW) and chemical formula.

Antibiotic name	Class/subclass	Abbreviation	MW	Chemical formula
Amoxicillin	Penicillins	AMX	365	C <sub>16</sub> H <sub>19</sub> N <sub>3</sub> O <sub>5</sub> S
Azithromycin	Macrolide	AZM	749	C <sub>38</sub> H <sub>72</sub> N <sub>2</sub> O <sub>12</sub>
Cefaclor	Cephalosporins	CEC	368	C <sub>15</sub> H <sub>14</sub> ClN <sub>3</sub> O <sub>4</sub> S
Cefalexin	Cephalosporins	LEX	347	C <sub>16</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub> S
Cefotaxime	Cephalosporins	CTX	456	C <sub>16</sub> H <sub>17</sub> N <sub>5</sub> O <sub>7</sub> S <sub>2</sub>
Chloramphenicol	Chloramphenicol derivatives	CAP	323	C <sub>11</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>5</sub>
Chlortetracycline	Diaminopyrimidines	CTC	479	C <sub>22</sub> H <sub>23</sub> ClN <sub>2</sub> O <sub>8</sub>
Ciprofloxacin	Fluoroquinolones	CIP	331	C <sub>17</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>
Clarithromycin	Macrolide	CTM	748	C <sub>38</sub> H <sub>69</sub> NO <sub>13</sub>
Clindamycin	Others	CLI	425	C <sub>18</sub> H <sub>33</sub> ClN <sub>2</sub> O <sub>5</sub> S
Cloxacillin	Penicillins	CXA	436	C <sub>19</sub> H <sub>18</sub> ClN <sub>3</sub> O <sub>5</sub> S
Doxycycline	Diaminopyrimidines	DC	463	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>8</sub>
Enoxacin	Fluoroquinolones	ENX	320	C <sub>15</sub> H <sub>17</sub> FN <sub>4</sub> O <sub>3</sub>
Enrofloxacin	Fluoroquinolones	EFL	359	C <sub>19</sub> H <sub>22</sub> FN <sub>3</sub> O <sub>3</sub>
Erythromycin	Macrolide	ERY	734	C <sub>37</sub> H <sub>67</sub> NO <sub>13</sub>
Lincomycin	Lincosamides	LIN	407	C <sub>18</sub> H <sub>34</sub> N <sub>2</sub> O <sub>6</sub> S
Lomefloxacin	Fluoroquinolones	LOM	351	C <sub>17</sub> H <sub>19</sub> F <sub>2</sub> N <sub>3</sub> O <sub>3</sub>
Metronidazole	Nitroimidazoles	MTZ	171	C <sub>6</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>
Norfloxacin	Fluoroquinolones	NOR	319	C <sub>16</sub> H <sub>18</sub> FN <sub>3</sub> O <sub>3</sub>
Ofloxacin	Fluoroquinolones	OFL	361	C <sub>18</sub> H <sub>20</sub> FN <sub>3</sub> O <sub>4</sub>
Oxytetracycline	Diaminopyrimidines	OTC	460	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>9</sub>
Penicillin G	Penicillins	PEN G	334	C <sub>16</sub> H <sub>18</sub> N <sub>2</sub> O <sub>4</sub> S
Penicillin V	Penicillins	PEN V	350	C <sub>16</sub> H <sub>18</sub> N <sub>2</sub> O <sub>5</sub> S
Roxithromycin	Macrolide	RTM	837	C <sub>41</sub> H <sub>76</sub> N <sub>2</sub> O <sub>15</sub>
Spiramycin	Macrolide	SPM	843	C <sub>43</sub> H <sub>74</sub> N <sub>2</sub> O <sub>14</sub>
Sulfachloropyridazine	Sulfonamides	SCP	285	C <sub>10</sub> H <sub>9</sub> ClN <sub>4</sub> O <sub>2</sub> S
Sulfadiazine		SDZ	250	C <sub>10</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub> S
Sulfadimethoxine		SDM	310	C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> O <sub>4</sub> S
Sulfamethazine		SMZ	278	C <sub>12</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub> S
Sulfamethoxazole	Sulfonamides	SMX	253	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S
Sulfapyridine	Sulfonamides	SPD	249	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub> S
Sulfasalazine	Sulfonamides	SSZ	398	C <sub>18</sub> H <sub>14</sub> N <sub>4</sub> O <sub>5</sub> S
Sulfathiazole	Sulfonamides	STZ	255	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> S <sub>2</sub>
Tetracycline	Diaminopyrimidines	TC	444	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>8</sub>
Trimethoprim	Diaminopyrimidines	TMP	290	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>
Tylosin	Macrolide	TYL	916	C <sub>46</sub> H <sub>77</sub> NO <sub>17</sub>

sulfonamides, quinolones, tetracyclines, macrolides and others) have been frequently detected in sewage, activated sludge, digested sludge and effluents (Table 1). Among these antibiotics, tetracyclines were the most commonly used, followed by sulfonamides and macrolides that accounted for approximately 90% of the total antibiotics used in the United Kingdom and more than 50% in Korea and Denmark (Kim et al., 2011). Verlicchi et al. (2012) found the most commonly investigated compounds were trimethoprim, sulfamethoxazole, erythromycin and ciprofloxacin in Italy. The highest

absolute concentrations were found for ofloxacin (32 µg L<sup>-1</sup>), roxithromycin (17 µg L<sup>-1</sup>) and ciprofloxacin (14 µg L<sup>-1</sup>).

Other antibiotics exhibiting measured concentrations greater than 10 µg L<sup>-1</sup> are: sulfapyridine (12.4 µg L<sup>-1</sup>), trimethoprim (10.5 µg L<sup>-1</sup>) and erythromycin (10.2 µg L<sup>-1</sup>). The highest average antibiotic concentrations were found for ofloxacin and sulfadiazine (5.1 µg L<sup>-1</sup>), followed by sulfapyridine (3.3 µg L<sup>-1</sup>) and cefalexim (3.2 µg L<sup>-1</sup>). No data were provided for enoxacin, lomefloxacin and spiramycin concentrations in the raw urban wastewater.



**Figure 1.** Scheme showing possible sources and pathways for the occurrence of pharmaceutical residues in the aquatic environment (Heberer, 2002).

## OCCURRENCE OF ANTIBIOTICS

The occurrence of pharmaceuticals as contaminants in wastewater and in the aquatic environment has attracted increased attention. A high percentage of pharmaceuticals consumed by humans are excreted unchanged via urine and feces into the sewage. Ultimately, their residual amounts can reach ambient surface waters or groundwater. Occurrence of antibiotics in effluent from potential sources *viz.* hospitals, residential facilities, dairies and municipal wastewater are of prime concern (Figure 1). Most antibiotics from various sources will eventually enter in environmental compartments through different pathways. The excretion of incompletely metabolized antibiotics by humans and animals is the primary source of antibiotics in the environment. All kinds of manures, as another important carrier of veterinary antibiotics, their transfer and seepage from manures to soil, surface, and groundwater also contribute to the environmental release of antibiotics (Kim and Carlson, 2005; Kim et al., 2005; Burkhardt et al., 2005; Kay et al., 2005).

Other sources include the disposal of unused antibiotics and waste from pharmaceutical manufacturing processes. Residential (private residences, dormitories, hotels and residential care facilities) and commercial facilities (including hospitals) are known contributors of antibiotics to municipal wastewater (Guiliani et al., 1996; Guardabassi et al., 1998; Hartmann et al., 1998; Alder et

al., 2003). Apart from these, further potential contributors of antibiotics to surface and ground water are effluent from wastewater treatment plants (WWTPs) (Golet et al., 2002; Hirsch et al., 1999; McQuillan et al., 2002; Alder et al., 2003) and industrial facilities (including pharmaceutical plants) (Guardabassi et al., 1998), and surface run-off from concentrated animal feeding operations (CAFOs) (Campagnolo and Rubin, 1998; Hirsch et al., 1999). Various antibiotics have been frequently detected in groundwater (Barnes et al., 2008; Batt et al., 2006a), drinking water (Focazio et al., 2008), surface water (Hirsch et al., 1999; Yang et al., 2011), sediment (Zhou et al., 2011) and agricultural lands (Hu et al., 2010; Karci and Balcioglu, 2009). In the municipal WWTPs, sulfamethoxazole, trimethoprim, norfloxacin, ofloxacin and anhydro erythromycin were the predominant antibiotics in the effluents, while in the sludge oxytetracycline, tetracycline, ofloxacin and norfloxacin were dominant with much higher concentrations than the others (Zhou et al., 2013).

Ye et al. (2004) found the largest number of antibiotics in its source water that is downstream of multiple wastewater plants and agricultural non-point discharge. Overall, fluoroquinolones were the most frequently detected antibiotics in source waters followed by sulfonamides, lincomycin, tetracyclines and macrolides (USP, 2003). Municipal wastewater had detections of sulfamethoxazole, trimethoprim, ciprofloxacin and ofloxacin, with four of six samples having at least one anti-

biotic present and 3 having 3 or more. The relatively high concentrations (up to 35,500 ng L<sup>-1</sup>) of ofloxacin found in hospital and residential effluent may be of concern due to potential genotoxic effects and development of antibiotic resistance. The results from a study in South China showed the presence of sulfamethoxazole, norfloxacin, ofloxacin, anhydro erythromycin and trimethoprim were most frequently detected in the WWTPs wastewaters. Twenty-one antibiotics were found in the sewage from the two WWTPs at the concentrations up to 5800 ng g<sup>-1</sup>, with tetracycline, oxytetracycline, norfloxacin and ofloxacin being the predominant antibiotics (Zhou et al., 2013).

The  $\beta$ -lactams, despite that they account for the highest proportion (50-70%) of the total human use antibiotic consumption, their occurrence was not detected frequently due to their unstable property (Kummerer, 2009). No  $\beta$ -lactams have been found in sludge samples all over the world despite their wide application in human and veterinary medicine (Minh et al., 2009), mainly due to their unstable characteristics and poor adsorption onto sludge. Golet et al. (2001) analyzed fluoroquinolone antibiotics in primary and tertiary wastewater effluents in Switzerland. In these samples, ciprofloxacin and norfloxacin occurred at concentrations between 249 and 405 ng L<sup>-1</sup> and from 45 to 120 ng L<sup>-1</sup>, respectively. Sacher et al. (2001) reported the occurrence of sulfamethoxazole (up to 410 ng L<sup>-1</sup>) and hydroerythromycin (up to 49 ng L<sup>-1</sup>) in groundwater samples in Germany. Holm et al. (1995) found residues of different sulfonamides at high concentrations in groundwater samples collected down gradient of a landfill in Grinsted, Denmark.

The concentration of antibiotics in the Yamuna River (India) varied from not detected to 13.75  $\mu$ g L<sup>-1</sup> (ampicillin) for the compounds investigated. Ampicillin had the maximum concentration in wastewater influents (104.2 $\pm$ 98.11  $\mu$ g L<sup>-1</sup>) and effluents (12.68 $\pm$ 8.38  $\mu$ g L<sup>-1</sup>). The fluoroquinolones and cephalosporins had lower concentrations (Mutiyaar and Mittal, 2014). In another study from Patancheru, Hyderabad (India), it was reported that WWTP effluents have the highest levels of ciprofloxacin (CIP) antibiotics residues (up to 31,000  $\mu$ g L<sup>-1</sup>) (Larsson et al., 2007). Later on, Fick et al. (2009) reported that water bodies receiving WWTP effluents often have high pharmaceutical residues as high peak concentration of antibiotics residues (up to 14,000  $\mu$ g L<sup>-1</sup>) from surface, groundwater, and drinking water of Hyderabad area. Hirsch et al. (1999) in various sewage, surface and groundwater samples in Germany did not detect penicillins or tetracyclines because the penicillins are easily hydrolyzed and tetracyclines readily precipitate with cations such as calcium and accumulate in sewage sludge or sediments (Daughton and Ternes, 1999; Stuer-Lauridsen et al., 2000).

The hospitals are primary contributor of antibiotics to municipal wastewater. Antibiotic occurrence was less pronounced in residential wastewater than in hospital

effluent. Hartmann et al. (1998) detected between 3 to 87  $\mu$ g L<sup>-1</sup> of the fluoroquinolone antibiotic ciprofloxacin in hospital effluents. Brown et al. (2006) reported that 58% of samples, out of 26 had at least one antibiotic present while 25% had three or more. Effluents discharged from hospitals showed the presence of sulfamethoxazole, trimethoprim, ciprofloxacin, ofloxacin, lincomycin and penicillin G. At the residential sampling sites, ofloxacin was found in effluent from assisted living and retirement facilities, while the student dormitory had no detection. Only lincomycin was detected in dairy effluent (in 2 out of 8 samples, at 700 and 6600 ng L<sup>-1</sup>). Brown et al. (2006) also reported lincomycin antibiotic which is commonly used to treat foot warts and mastitis in lactating cows, was detected in two out of eight analyzed dairy effluent samples at concentrations of 700 and 6600 ng L<sup>-1</sup>.

The ample occurrence of antibiotics in the environment could affect aquatic and terrestrial organisms (Costanzo et al., 2005; Kotzerke et al., 2008; Liu et al., 2009), alter microbial activity and community composition (Underwood et al., 2011), and lead to prevalence of bacterial resistance to antibiotics (LaPara et al., 2011; Su et al., 2012; Tao et al., 2010). Although, the occurrence level of individual antibiotics may be low, but the presence of a broad range of antibiotics in some source water suggests that the overall effect of the antibiotics as a contaminant group should not be underestimated. In order to reduce the negative impacts on the environment and human health, it is necessary to understand the input sources for various classes of antibiotics. Therefore, the fate of pharmaceutical compounds in wastewater and other water systems should be properly investigated, including monitoring ambient concentrations in STPs and receiving water bodies.

## CONCENTRATION OF ANTIBIOTICS

Based on the literature reviewed, huge variability in the concentration of each compound in both raw urban influents as well as in secondary effluent was observed. Mutiyaar and Mittal (2014) measured the mean residues concentration in influents was 104.2  $\mu$ g L<sup>-1</sup> for AMP, 20.1  $\mu$ g L<sup>-1</sup> for CIP, 2.7  $\mu$ g L<sup>-1</sup> for gatifloxacin (GAT), 22.5  $\mu$ g L<sup>-1</sup> for SPA and 3.4  $\mu$ g L<sup>-1</sup> for cefuroxime (CEF). For effluents, the mean antibiotics residues concentration was 12.68  $\mu$ g L<sup>-1</sup> for AMP, 8  $\mu$ g L<sup>-1</sup> for CIP, 1.22  $\mu$ g L<sup>-1</sup> for GAT, 0.14  $\mu$ g L<sup>-1</sup> for sparfloxacin SPA, and 0.22  $\mu$ g L<sup>-1</sup> for CEF. These are higher levels of antibiotics in wastewater than previously reported by others studies (Yang et al., 2005; Xu et al., 2007; Heidler and Halden, 2008; Loganathan et al., 2009) from different parts of the world, but lesser than those reported by Li et al. (2008). Xu et al. (2007) and Heidler and Halden (2008) reported similar levels of antibiotics to those reported here (10.8  $\mu$ g L<sup>-1</sup> in treated wastewater). Yang et al. (2005) reported concentrations of pharmaceutical resi-

dues around  $1.1 \mu\text{g L}^{-1}$ . Li et al. (2008) reported  $389 \text{ ng L}^{-1}$  of penilloic acid, a degradation product of penicillin G, with  $153 \mu\text{g L}^{-1}$  of the parent compound also present. High concentrations of oxytetracycline ( $238\text{--}1680 \text{ ng g}^{-1}$ ) and tetracycline ( $117\text{--}1650 \text{ ng g}^{-1}$ ), ofloxacin ( $1500\text{--}5800 \text{ ng g}^{-1}$ ) and norfloxacin ( $1820\text{--}5610 \text{ ng g}^{-1}$ ) were found in sludge samples in China (Zhou et al., 2013; Gao et al., 2012; Jia et al., 2012) and doxycycline at a concentration of  $1500 \text{ ng/g}$  in Swedish sludge samples (Lindberg et al., 2005). CTC was found in 2.4% of the 84 surface water samples with the maximum concentration of  $0.69 \mu\text{g L}^{-1}$  by Kolpin et al. (2002). Gulkowska et al. (2007) detected tetracycline, erythromycin, norfloxacin and trimethoprim in Hong Kong coastal waters. Managaki et al. (2007) reported that the veterinary antibiotics, e.g. erythromycin, trimethoprim and various sulfonamides, were detected in the Mekong Delta in Vietnam. The highest concentrations of penicillin V detected in influent and effluent were up to  $13800$  and  $2000 \text{ ng L}^{-1}$  (Watkinson et al., 2009), respectively.

Zhou et al. (2013) reported antibiotic concentration in two wastewater treatment plants in May and November and found that out of 50 target compounds, 19 and 15 antibiotics were quantified with concentrations ranging from  $4.58 \pm 0.03$  to  $942 \pm 15.7$  and  $1.14 \pm 0.32$  to  $695 \pm 23.7 \text{ ng L}^{-1}$  in the influent and final effluent of plant A, respectively. For plant B, 18, 16 and 15 were detected at concentrations ranging from  $3.22 \pm 0.76$  to  $861 \pm 30.6 \text{ ng L}^{-1}$  in the influent, from  $3.28 \pm 0.53$  to  $277 \pm 3.48 \text{ ng L}^{-1}$  in the secondary effluent, and from  $3.20 \pm 0.06$  to  $101 \pm 1.04 \text{ ng L}^{-1}$  in the final effluent after disinfection, respectively. Trimethoprim was found in the concentrations ranging from  $72.3 \pm 0.83$  (plant B in May) to  $162 \pm 9.09 \text{ ng L}^{-1}$  (plant A in November) in the influents, and ranging from  $31.1 \pm 0.74$  (plant B in May) to  $64 \pm 1.95 \text{ ng L}^{-1}$  (plant A in November) in the effluents. Lincomycin was also detected in the two plants, with the concentrations ranging from  $44.2 \pm 0.10$  (plant B in November) to  $129 \pm 2.96 \text{ ng L}^{-1}$  (plant A in May) in the influents, and ranging from ND (not detected) (plant A in November and plant B in November) to  $53.9 \pm 4.31 \text{ ng L}^{-1}$  (plant B in May) in the effluents. Chloramphenicol was only found in plant A with the concentrations below  $22.1 \pm 0.34 \text{ ng L}^{-1}$  in the influents (in May) and below  $5.8 \pm 0.42 \text{ ng L}^{-1}$  (in November) in the effluents. Whereas in the case of suspended solids and sludge, sulfonamides and trimethoprim were found with concentrations below  $36.4 \pm 1.25 \text{ ng g}^{-1}$  (sulfamethoxazole). Five tetracyclines and 7 fluoroquinolones were detected in concentrations ranging from  $5.61 \pm 1$  (methacycline) to  $1680 \pm 16.7 \text{ ng g}^{-1}$  (oxytetracycline) and from  $1.72$  (pefloxacin) to  $5800 \pm 157 \text{ ng g}^{-1}$  (ofloxacin), respectively. Tetracyclines and fluoroquinolones were the predominant antibiotics detected in the sludge, and they contributed above 90% of the total loads of antibiotics in the dewatered sludge. Three macrolides found in the wastewater were quantified in the suspended solids and sludge, with the

concentrations ranging from  $4.64 \pm 0.68$  (roxithromycin) to  $147 \pm 6.01 \text{ ng g}^{-1}$  (anhydroerythromycin) (Zhou et al., 2013).

Higher concentration levels of antibiotics have been reported in various rivers throughout the globe as per literature as follows:  $11.92 \mu\text{g L}^{-1}$  of sulfamethoxazole in the Llobregat river in NE Spain (Munoz et al., 2009),  $10 \mu\text{g L}^{-1}$  of CIP in the Arc river in France (Feitosa-Felizzola and Chiron, 2009),  $1.3 \mu\text{g L}^{-1}$  of CIP in the Brisbane river Australia (Watkinson et al., 2009) and  $80 \mu\text{g L}^{-1}$  of ERY in the Duhan river in Taiwan (Lin and Tsai, 2009). Yamuna River in India is one of the most contaminated river stretches in India. It receives around 3,000 million liter per day, that is, MLD of sewage from various drains. Concentration of amoxicillin in the untreated raw sewage at the STP located in Delhi varied from ND to  $172.6 \text{ ng L}^{-1}$  and after treatment; it varied from ND to  $62.5 \text{ ng L}^{-1}$  (Mutiyaar and Mittal, 2013). The influent loadings varied from  $301$  to  $11,462 \text{ g day}^{-1}$  for the different antibiotics and  $15.4\text{--}1,395 \text{ g day}^{-1}$  were released in the effluents (Mutiyaar and Mittal, 2014). The estimated loadings were  $1,395 \text{ g day}^{-1}$  for AMP,  $880 \text{ g day}^{-1}$  for CIP,  $134 \text{ g day}^{-1}$  for GAT,  $15.4 \text{ g day}^{-1}$  for SPA, and  $24.2 \text{ g day}^{-1}$  for CEF, and the total loading from these compounds is  $2,428 \text{ g day}^{-1}$ . Similarly, high concentrations of sulfonamides ( $20 \times 10^{-3} \text{ ng/ml}$ ) have been found in pig farm wastewater, and detection of sulfamethazine has been suggested to serve as a marker for livestock-source contamination in Vietnam (Managaki et al., 2007).

## FATE OF ANTIBIOTICS IN THE SOIL

After entering antibiotics in agricultural soil, several interrelated processes were involved, including degradation (Kreuzig and Holtge, 2005), adsorption (Rabolle and Spliid, 2000), transport (leaching and runoff) (Blackwell et al., 2007), and plant uptake (Migliore et al., 1996; Kumar et al., 2005a; Boxall et al., 2006; Dolliver et al., 2007). The active ingredients in the upper soil layer might either accumulate in soil or be absorbed by crops, or be readily available for transport into surface and groundwater through leaching and overland flow runoff (Jongbloed and Lenis, 1998). All dynamic processes of antibiotics in soils are closely interrelated and driven by crops, soil microorganisms, water and anthropogenic activities, which will ultimately determine the spatial-temporal distribution and environmental impacts of antibiotics. Tetracycline could persist in soil for over 1 year (Zuccato et al., 2000), and only a moderate degradation of various tetracycline occurred within 180 days (Hamscher et al., 2002). Kolpin et al. (2002) detected about  $9.5 \mu\text{g kg}^{-1}$  CTC in the soil surface, two days before sampling manure with animal slurry. Many countries had also detected the antibiotic residues in soils (Boxall, 2004; Hamscher et al., 2005; Martinez-Carballo et al., 2007).

## ANTIBIOTIC RESISTANCE

The emergence of resistance of bacteria to antibiotics is a common phenomenon. Antibiotic resistance is a form of drug resistance whereby sub-populations of a microorganism, usually strains of a bacterial species, are able to survive after exposure to one or more antibiotics; pathogens resistant to multiple antibiotics are considered multidrug resistant (MDR) or, more colloquially, superbugs. It is a serious and hasty growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concerns of the 21<sup>st</sup> century, in particular as it pertains to pathogenic organisms. A World Health Organization report released in April 30, 2014 states, "This serious threat is no longer a prediction for the future, it is happening right now in every region of the world and has the potential to affect anyone, of any age, in any country. Antibiotic resistance, when bacteria change, so antibiotics no longer work in people who need them to treat infections, is now a major threat to public health. Genes for resistance to antibiotics, like the antibiotics themselves, are ancient. However, the increasing prevalence of antibiotic-resistant bacterial infections seen in clinical practice stems from antibiotic use both within human medicine and veterinary medicine. Any use of antibiotics can increase selective pressure in a population of bacteria to allow the resistant bacteria to thrive and the susceptible bacteria to die off. As resistance towards antibiotics becomes more common, a greater need for alternative treatments arises.

In medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics (WHO, 2002). In some countries, antibiotics are sold over the counter without a prescription, which also leads to the creation of resistant strains. Wastewater from hospitals and intensive farming facilities is probably a major source of pathogenic and antibiotic-resistant organisms and antibiotic-resistance genes that are released into the environment. Other practices contributing to resistance include antibiotic use in livestock feed to promote faster growth (Ferber, 2002; Mathew et al., 2007). Household use of antibacterial in soaps and other products, although not clearly contributing to resistance, is also discouraged. Unsafe practices in the pharmaceutical manufacturing industry can also contribute towards the likelihood of creating antibiotic resistant strains (Larsson and Fick, 2009). The procedures and clinical practice during the period of drug treatment are frequently flawed; usually no steps are taken to isolate the patient to prevent re-infection or infection by a new pathogen. Resistance may take the form of biodegradation of pharmaceuticals, such as sulfamethazine degrading soil bacteria introduced to sulfamethazine through medicated pig feces (Topp et al., 2013). Along with antibiotic waste, resistant bacteria follow, thus introducing antibiotic resistant bacteria into the environment. As bacteria replicate quickly, the resistant bacteria that enter the environment replicate their



**Figure 2.** Bacterial culture in the media for testing antibiotic resistance.

resistance genes as they continue to divide. In addition, bacteria carrying resistance genes have the ability to spread those genes to other species via horizontal gene transfer. Therefore, even if the specific antibiotic is no longer introduced into the environment, antibiotic resistance genes will persist through the bacteria that have since replicated without continuous exposure.

The antimicrobial resistance may be evidenced by dilution or disk diffusion methods. Figure 2 shows a test in which the strain on the right is resistant to most antibiotics tested.

### Mechanisms of antibiotic resistance

Antibiotic resistance can be a result of mutation or horizontal gene transfer. The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

1. Drug inactivation or modification: for example, enzymatic deactivation of penicillin G in some penicillin resistant bacteria through the production of  $\beta$ -lactamases.
2. Alteration of target site: for example, alteration of PBP (the binding target site of penicillins), or production of an additional PBP2a produced by strains of *S. aureus* methicillin resistant (MRSA).
3. Alteration of metabolic pathway: for example, some sulfonamide resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulfonamides, instead, like mammalian cells, they turn to using preformed folic acid.
4. Reduced drug accumulation: by decreasing drug permeability (for example quinolones) or increasing active efflux (pumping out) of the drugs across the cell surface (for example tetracycline).

### Antibiotic resistant bacteria

Antibiotics are of concern due to potential genotoxic

effects, disruption of aquatic ecology, promotion of antibiotic resistance, complications surrounding development of water reuse technologies, and possibly even increased human health risks (Daughton and Ternes, 1999). The widespread and often inappropriate administration of antibiotics in livestock, pets and humans has been shown to result in the development of antibiotic-resistant bacteria and is generally accepted to be a primary pathway for their proliferation in the environment. Although the concentration of antibiotics residue in the environment is very low, usually at the  $\text{ng L}^{-1}$  to  $\mu\text{g L}^{-1}$  level in water and the  $\mu\text{g kg}^{-1}$  to  $\text{mg kg}^{-1}$  level in soil, they still draw great attention all over the world because antibiotics and their transformation products may result in the development, maintenance, transfer or spread of antibiotic resistant bacteria and antibiotic resistance genes in the long term and have serious impacts on the ecosystem (Zhang et al., 2009).

Intense use of antibiotics in agricultural production may lead to the contamination of surface and groundwater by antibiotic-resistant bacteria. The spread of antibiotic-resistant bacteria in the environment may compromise the ability to treat microbial infections and has become an increasing challenge to public health. Today antibiotic resistance is a global health problem that involves all major pathogens and antimicrobial drugs (Capita and Alonso-Calleja, 2013) and is now classified as a global pandemic (EASAC, 2007). Discharge of antibiotics to the environment from the wastewater has been linked to the development of various resistant bacterial strains (Kummerer, 2004). Water discharged into water bodies from municipal sewage treatment plants may contain significant concentrations of the genes that make bacteria antibiotic-resistant. Timothy et al. (2011) explained that antibiotic resistant bacteria are abundant in the sewage that enters municipal wastewater treatment plants. Treatment is intended to kill the bacteria, and it removes many of the bacterial genes that cause antibiotic resistance. However, genes or bacteria may be released in the effluent from the plant.

The intensive use of antibiotics within primary production is the main reason for the spread of antimicrobial resistance throughout the food chain (Capita and Alonso-Calleja, 2013); with tetracycline being one of the most used antimicrobial agents in agricultural production (Kools et al., 2008). The use of antibiotics in agricultural production has resulted in a high level of antibiotic-resistant bacteria in animal waste (Parveen et al., 2006; Sapkota et al., 2007). Tetracycline resistance has been seen in 80% of *Escherichia coli* isolated from 90 Canadian farms (Varga et al., 2008) and in the range of 20–50% of *E. coli* isolated from farms in Florida (Parveen et al., 2006). As a consequence, the large-scale application of animal waste onto agricultural land releases large quantities of antibiotics, resistance genes, resistant bacteria and pathogenic bacteria into the soil environment (Kjaer et al., 2007; Chee-Sanford et al.,

2009), counteracting the positive effects of spreading animal waste on agricultural land. The use of antibiotics in agricultural production has also led to the detection of resistant bacteria in surface water in the US and Mexico where it has been associated with agricultural activities (Lugo-Melchor et al., 2010; Sapkota et al., 2007). In a study, a total of 767 *E. coli* isolates were tested regarding their resistance to 24 different antibiotics. The highest resistance rates were found in *E. coli* strains of a sewage treatment plant which treats not only municipal sewage but also sewage from a hospital. Among the antimicrobial agents tested, the highest resistance rates in the penicillin group were found for ampicillin (up to 18%) and piperacillin (up to 12%); in the cephalosporin group for cefalothin (up to 35%) and cefuroxime-Axetil (up to 11%); in the group of quinolones for nalidixic acid (up to 15%); and for trimethoprim/sulfamethoxazole (up to 13%) and for tetracycline (57%) (Reinthaler et al., 2013). Similarly, developments of antibiotic-resistance bacteria (Middleton and Salierno, 2012; Shah et al., 2012) and risk to aquatic organisms (Zhang et al., 2012) have been correlated with pharmaceutical residues presence in wastewater from inadequate wastewater treatment systems. Samples from surface water sites near wastewater treatment plants in Australia had a significant increase of antibiotic resistant *E. coli* (Watkinson et al., 2007).

Kummerer (2004) reported the development of various resistant bacterial strains associated with discharges of antibiotics. Kim et al. (2008) and Oetken et al. (2004) reported death and decline in reproduction of standard test organisms like *V. fischeri*, *D. magna*, *M. macrocopa*, *O. latipes* and some invertebrates. Alighardashi et al. (2009) reported acute sensitivity of sludge bacteria to erythromycin causing floc disintegration and breakage of filaments. Genotoxic substances are often mutagenic and carcinogenic and are, therefore, potentially problematic in the development of antibiotic resistant organisms. The fluoroquinolone antibiotics were shown to display high genotoxicity at concentrations ( $3000$  to  $87,000 \text{ ng L}^{-1}$ ) detected in hospital effluent (Hartmann et al., 1998). Fluoroquinolone antibiotics have been shown to exert genotoxic effects on the genetically modified bacterial strain, *Salmonella Typhimurium*, at concentrations as low as  $5000 \text{ ng/L}$  for norfloxacin and  $25,000 \text{ ng L}^{-1}$  for ciprofloxacin (Hartmann et al., 1998). Fluoroquinolones are more toxic to prokaryotic than eukaryotic organisms and do not appear to pose an acute human genotoxic risk at concentrations found in hospital effluent. However, there may be concerns about their impact on aquatic microbial populations. The sewers downstream from a hospital increased prevalence of bacteria resistant to oxytetracycline, whereas sewers downstream from a pharmaceutical plant showed an increased pervasiveness of bacteria resistant to multiple drugs, including sulfamethoxazole. These results lend credence to the concern that antibiotic-resistant bacteria might develop from long-term environmental exposure to relatively low

concentrations of antibiotics (Guardabassi et al., 1998).

### Some examples of the resistant pathogens

1. The most serious concern with antibiotic resistance is that some bacteria have become resistant to almost all of the easily available antibiotics. These bacteria are able to cause serious disease and this is a major public health problem. Important examples are MRSA, vancomycin-resistant Enterococcus (VRE), multidrug resistant *Mycobacterium tuberculosis* (MDR-TB), *Acinetobacter baumannii*, KPC strains, *Pseudomonas aeruginosa* etc.

2. Antibiotics such as penicillin and erythromycin, which used to have a high efficacy against many bacterial species and strains, have become less effective, due to the increased resistance of many bacterial strains (Pearson, 2007).

3. *S. aureus* is one of the major resistant pathogens. It was one of the earlier bacteria in which penicillin resistance was found in 1947, just four years after the drug was mass produced. *S. aureus* infections in the US are resistant to penicillin, methicillin, tetracycline and erythromycin. The MRSA is the most frequently identified antimicrobial drug resistant pathogen in US hospitals. The United States in 2007 had reported seven cases of vancomycin-resistant *Staphylococcus aureus* (VRSA) infection, a serious development that has healthcare providers fearful of losing ground in their attempt to control the spread of *S. aureus*.

4. *Streptococcus pyogenes* infections can usually be treated with many different antibiotics. Strains of *S. pyogenes* resistant to macrolide antibiotics have emerged; however, all strains remain uniformly susceptible to penicillin (Albrich et al., 2004). Resistance of *Streptococcus pneumoniae* to penicillin and other  $\beta$ -lactams is increasing worldwide. *S. pneumoniae* is responsible for pneumonia, bacteremia, otitis media, meningitis, sinusitis, peritonitis and arthritis (Albrich et al., 2004).

5. *Clostridium difficile* is a nosocomial pathogen that causes diarrheal disease in hospitals worldwide (Gerding et al., 1995; McDonald, 2005). Clindamycin resistant *C. difficile* was reported as the causative agent of large outbreaks of diarrheal disease in hospitals in New York, Arizona, Florida and Massachusetts (Johnson et al. 1995). *C. difficile* strains resistant to fluoroquinolone antibiotics, such as ciprofloxacin and levofloxacin, were also reported in North America in 2005 (Loo et al., 2005).

6. Tuberculosis (TB) is increasing across the globe, especially in developing countries, over the past few years. Its resistant to antibiotics is called MDR TB (Multidrug Resistant TB - *M. tuberculosis* strain that is resistant to at least isoniazid and rifampin). Globally, MDR TB causes 150,000 deaths annually (Edward et al., 2013). It was considered one of the most prevalent diseases, and did not have a cure until the discovery of

streptomycin by Selman Waksman in 1943 (Herzog, 1998). However, the bacteria soon developed resistance. Since then, drugs such as isoniazid and rifampin have been used. *M. tuberculosis* develops resistance to drugs by spontaneous mutations in its genomes. Resistance of *M. tuberculosis* to isoniazid, rifampin and other common treatments has become an increasingly relevant clinical challenge.

For a long time, it has been thought that, for a microorganism to become resistant to an antibiotic, it must be in a large population. However, recent findings show that there is no necessity of large populations of bacteria for the appearance of antibiotic resistance. The leaching of bacteria from agricultural soils is a problem in well-structured soils where preferential flow through soil macropores results in rapid downward transport.

### IMPACTS ON SOIL MICROORGANISMS AND SOIL ENZYME ACTIVITY

The detection of antibiotic residues (even at subnanogram per liter) is alarming for ecosystem sustainability. These compounds are specially engineered to show their effect at trace levels. There are no reports on direct effect on human beings from contaminated water but the effects on other organisms have been documented. Long-term accumulation of persistent antibiotics and their metabolites in agro-ecosystems are bioactive and potentially hazardous to soil microorganisms and crops, particularly bacteria (Bagner et al., 2000). Degradation products of antibiotics exhibited toxicity to microorganisms (Ge et al., 2010). Residual antibiotics in the soils help in developing antibiotic resistant microbial populations (Witte, 1998; Morris and Masterton, 2002), microbial activity (Jjemba, 2002a, b), and alter soil microbial constitution and functions. There is limited information available on the direct effects of these drugs on soil biota, particularly those living in rhizosphere.

Antibiotics act as an ecological factor in the environment that could potentially influence microbial communities. Ding and He (2010) summarized the effect of antibiotics in the environment on microbial populations. The effects include phylogenetic structure alteration, resistance expansion, and ecological function disturbance in the micro-ecosystem and changes of microbial community structure upon addition of antibiotics in soil and water environment. Effects of antibiotics on ecological functions also include nitrogen transformation, methanogenesis, and sulfate reduction. Diao et al. (2004) reported that apramycin inhibited soil bacteria growth significantly. Tetracyclines, a group of natural and semisynthetic antibiotics inhibited bacterial protein synthesis. Hernando et al. (2006) showed adverse effects of pharmaceutical residues on bacteria, invertebrates and

algal populations in soils. The impacts of antibiotics on soil organisms will certainly modify enzyme activities and soil biochemical processes. The presence of tetracycline disturbed the structure of microbial communities and inhibited soil microbial activities in terms of urease, acid phosphatase, and dehydrogenase (Wei et al., 2009). The antibiotics, including CTC, TC, TYL, SMX, SMZ and trimethoprim, inhibited soil phosphatase activity whereas sulfonamides (SMX and SMZ) and trimethoprim inhibited the soil respiration (Liu et al., 2009). The OTC decreased the urease, sucrase, phosphatase, hydrogen peroxidase and microbial biomass nitrogen in rhizosphere of wheat, and increased the microbial biomass carbon (Yao et al., 2010). Besides deleterious effects on soil microflora and crop growth, antibiotics can provoke resistant pathogens through long-time exposure to the soil environment due to genetic variation and transfer. As novel persistent pollutants, antibiotics were included in limitations issued by the Food and Agriculture Organization (FAO) and the USA. High concentrations of antibiotics in the sludge would have negative impacts on the environment if the sludge were applied on agricultural land, including effects on soil microbial diversity, respiration and denitrification due their antimicrobial activities (Chander et al., 2005; Kotzerke et al., 2011; Zielesny et al., 2006).

## ENVIRONMENTAL RISK AND HEALTH HAZARDS

Antibiotics present in the sewage water could possibly have harmful ecotoxicological effects on aquatic organisms. Aquatic lives in the water bodies receiving treated sewage are exposed to a mixture of drug residues, not just single compounds. The presence of antibiotic residue in sewage, activated sludge, digested sludge and urban biosolids has become an increasingly recognized environmental risk (Zhang and Li, 2011). Residues of the antibiotics in the farm dust present a new source of health hazard for farmers (Hamscher et al., 2003). Hamscher et al. (2003) in Germany detected five different antibiotics in 90% of dust samples collected from the piggery of which total amounts were up to 12.5 mg kg<sup>-1</sup> dust.

The results suggested that the risks may arise from the inhalation of dust contaminated with antibiotics. Crops absorb antibiotics from polluted soils accumulated due to sewage irrigation or manure amended soils through fertilization (Dotaniya et al. 2014). High level accumulation of antibiotics in food crops may raise potential human health concerns through the food chain. Uptake varies for different antibiotics and it also depends on plant and their species. Some antibiotics, for example, TYL, could not be absorbed by corn, onion and cabbage because of their high molecular weight (Kumar et al., 2005a). However, since little is known regarding environmentally safe levels for antibiotics in wastewater or surface waters (Golet et al., 2002), more research is needed to quantify the risk so

that appropriate action can be taken to mitigate harmful effects or alternately, redirect efforts and limited resources.

## CONCLUSION

Environmental contamination by antibiotics has become an increasingly serious problem worldwide, which poses great risks to ecosystems and human health. Most of the antibiotics detected in source water are not detected in finished water or are present at a much lower level, if detected. It indicates their partial removal during conventional treatment processes or chemical transformation during disinfection. The incomplete removal of some antibiotics, such as ciprofloxacin, is of concern due to their unknown health effects if they do persist in finished waters even at ng L<sup>-1</sup> levels. Currently, there is lack of understanding on ecotoxic impacts of antibiotics on soil ecology, particularly growth, community structure, function and diversity. In addition, some beneficial rhizosphere microorganisms, like *Arbuscular mycorrhizal*, fungus and *Rhizobium* have not been investigated till now. So, future research will be focused on how these antibiotics persist and transform during water treatment processes. More research is needed to evaluate the potential impacts of these antibiotics with high mass loads on the receiving environment following the discharge of effluents and disposal of sludge.

## Conflict of interest

The author(s) have not declared any conflict of interests.

## REFERENCES

- Albrich WC, Monnet DL, Harbarth S, Monnet DL, Harbarth S (2004). Antibiotic selection pressure and resistance in *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *Emerg. Infect. Dis.* 10(3):514-517.
- Alder AC, McArdell CS, Golet EM, Molnar EM, Kuch HM, Giger W (2003). Environmental exposure of antibacterial agents in hospital and municipal wastewater and river water in Switzerland [Abs.]. Proceedings of 3rd international conference on pharmaceuticals and endocrine disrupting chemicals in water, 19–21 March 2003, Minneapolis, MN. pp. 236-237.
- Alighardashi A, Pandolfi D, Potier O, Pons MN (2009). Acute sensitivity of activated sludge bacteria to erythromycin. *J. Hazard. Mater.* 172:685-692.
- Baguer AJ, Jensen J, Krogh PH (2000). Effects of the antibiotics oxytetracycline and TYL on soil fauna. *Chemosphere* 40:751–757.
- Barnes KK, Kolpin DW, Furlong ET, Zaugg SD, Meyer MT, Barber LB (2008). A national reconnaissance of pharmaceuticals and other organic wastewater contaminants in the United States-I groundwater. *Sci. Total Environ.* 402:192-200.
- Batt AL, Bruce IB, Aga DS (2006a). Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. *Environ. Pollut.* 142:295–302.
- Batt AL, Snow DD, Aga DS (2006b). Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA. *Chemosphere* 64:1963-1971.

- Berset JD, Kupper T, Etter R, Tarradellas J (2004) Consideration about the enantioselectivity transformation of polycyclic musks in wastewater, treated wastewater, and sewage sludge and analysis of their fate in a sequencing batch reactor plant. *Chemosphere* 57: 987-996
- Blackwell PA, Kay P, Boxall ABA (2007). The dissipation and transport of veterinary antibiotics in a sandy loam soil. *Chemosphere* 67:292-299.
- Boxall ABA (2004). The environmental side effects of medication: how are human and veterinary medicines in soils and water bodies affecting human and environmental health? *EMBO Rep.* 5:1110-1116.
- Boxall ABA, Johnson P, Smith EJ, Sinclair CJ, Stutt E, Levy L (2006). Uptake of veterinary medicines from soils into plants. *J. Agric. Food Chem.* 163(3):2288-2297.
- Brown KD, Kulis J, Thomson B, Chapman TH, Mawhinney DB (2006). Occurrence of antibiotics in hospital, residential, and dairy, effluent, municipal wastewater, and the Rio Grande in New Mexico. *Sci. Total Environ.* 366: 772-783.
- Burkhardt M, Stamm C, Waul C, Singer H, Muller S (2005). Surface runoff and transport of sulfonamide antibiotics and tracers on manured grassland. *J. Environ. Qual.* 34:1363-1371.
- Campagnolo ER, Rubin CS (1998). Report to the state of Iowa department of public health on the investigation of the chemical and microbial constituents of ground and surface water proximal to large-scale swine operations; 1998. October-December, Available at <http://www.factoryfarm.org/docs/CDCwaterqualityfinaldraft.doc>, Accessed on April 2014.
- Capita R, Alonso-Calleja C (2013). Antibiotic-resistant bacteria: a challenge for the food industry. *Crit. Rev. Food Sci. Nutr.* 53: 11-48.
- Chander Y, Kumar K, Goyal SM, Gupta SC (2005). Antibacterial activity of soil-bound antibiotics. *J. Environ. Qual.* 34:1952-1957.
- Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin Y-FF, Yannarell AC, Maxwell S, Aminov RI (2009). Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J. Environ. Qual.* 38: 1086-1108.
- Costanzo SD, Murby J, Bates J (2005). Ecosystem response to antibiotics entering the aquatic environment. *Mar. Pollut. Bull.* 51:218-223.
- Daughton CG, Ternes TA (1999). Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* 107:907-938.
- Deblonde T, Cossu-Leguille C, Hartemann P (2011). Emerging pollutants in wastewater: a review of the literature. *Int. J. Hyg. Environ. Health* 214:442-448.
- Diao XP, Sun YJ, Sun ZJ, Shen JZ (2004). Effects of Apramycin on microbial activity in different types of soil. *Ecol. Environ.* 13 (4):565-568.
- Ding C, He JZ (2010). Effect of antibiotics in the environment on microbial populations. *Appl. Microbiol. Biotechnol.* 87:925-941.
- Dolliver H, Kumar K, Gupta S (2007). Sulfamethazine uptake by plants from manure-amended soil. *J. Environ. Qual.* 36:1224-1230.
- Dotaniya ML, Saha JK, Meena VD, Rajendiran S, Coumar MV, Kundu S, Rao AS (2014). Impact of tannery effluent irrigation on heavy metal build up in soil and ground water in Kanpur. *Agrotechnology* 2(4):77.
- EASAC (2007). Tackling antibacterial resistance in Europe. European Academies Science Advisory Council, June 2007.
- Edward J, Septimus MD, FIDSA, FACP, FSHEA (2013). Antimicrobial resistance still poses a public health threat: a conversation with Edward et al. Clinical professor of internal medicine at Texas A&M health science center. Agency for Healthcare Research and Quality. 2013-04-17. Retrieved 2013-09-26.
- Fatta-Kassinos D, Meric S, Nikolaou A (2011). Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. *Anal. Bioanal. Chem.* 399:251-275.
- Feitosa-Felizzola J, Chiron S (2009). Occurrence and distribution of selected antibiotics in a small Mediterranean stream (Arc River, Southern France). *J. Hydrol.* 364:50-57.
- Ferber D (2002). Livestock Feed Ban Preserves Drugs' Power. *Sci.* 295(5552):27-28.
- Fick J, Soderstrom H, Linderberg RH, Phan C, Tysklind M, Larsson DGJ (2009). Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ. Toxicol. Chem.* 28:2522-2527.
- Focazio MJ, Kolpin DW, Barnes KK, Furlong ET, Meyer MT, Zaugg SD, Barber LB, Thurman ME (2008). A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States: II. Untreated drinking water resources. *Sci. Total Environ.* 402:201-216.
- Gao L, Shi Y, Li W, Niu H, Liu J, Cai Y (2012). Occurrence of antibiotics in eight sewage treatment plants in Beijing, China. *Chemosphere* 86:665-671.
- Ge LK, Chen JW, Zhang SY, Cai XY, Wang Z, Wang CL (2010). Photodegradation of fluoroquinolone antibiotic gatifloxacin in aqueous solutions. *China Sci. Bull.* 55(15):1495-1500.
- Gerding DN, Johnson S, Peterson LR, Mulligan ME, Silva J (1995). *Clostridium difficile*-associated diarrhea and colitis. *Infect. Control Hosp. Epidemiol.* 16 (8):459-477.
- Golet EM, Alder AC, Giger W (2002) Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. *Environ. Sci. Technol.* 36:3645-3651.
- Golet EM, Alder AC, Hartmann A, Ternes TA, Giger W (2001). Trace determination of Fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. *Anal. Chem.* 73:3632-3638.
- Guardabassi L, Petersen A, Olsen JE, Dalsgaard A (1998). Antibiotic resistance in *Acinetobacter* spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant. *Appl. Environ. Microbiol.* 64(9):3499-502.
- Guiliani F, Koller T, Wurgler FE, Widmer RM (1996). Detection of genotoxic activity in native hospital waste water by the umuC test. *Mutat. Res.* 368:49-57.
- Gulkowska A, He YH, So MK, Yeung LWY, Leung HW, Giesy JP, Lam PKS, Martin M, Richardson BJ (2007). The occurrence of selected antibiotics in Hong Kong coastal waters. *Mar. Pollut. Bull.* 54(8):1287-1293.
- Hamscher G, Pawekzick HT, Sczesny S, Nau H, Hartung J (2003). Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? *Environ. Health Perspect.* 111 (13):1590-1594.
- Hamscher G, Pawelzick HT, Hoper H, Nau H (2005). Different behavior of tetracyclines and sulfonamides in sandy soils after repeated fertilization with liquid manure. *Environ. Toxicol. Chem.* 24:861-868.
- Hamscher G, Sczesny S, Höper H, Nau H (2002). Determination of persistent tetracycline residues in soil fertilized with liquid manure by high performance liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Chem.* 74:1509-1518.
- Hartmann A, Alder A, Koller T, Widmer R (1998). Identification of fluoroquinolone antibiotics as the main source of umuC genotoxicity in native hospital wastewater. *Environ. Toxicol. Chem.* 17:377-382.
- Heidler J, Halden R (2008) Meta-analysis of mass balances examining chemical fate during wastewater treatment. *Environ. Sci. Technol.* 42:6324-6332.
- Hernando MD, Mezcuca M, Fernandez-Alba AR, Barcelo D (2006). Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters, and sediments. *Talanta* 69: 334-342.
- Herzog H (1998). History of Tuberculosis. *Respiration* 65(1):5-15.
- Hirsch R, Ternes TA, Haberer K, Kratz KL (1999). Occurrence of antibiotics in the aquatic environment. *Sci. Total Environ.* 225: 109-118.
- Hirsch R, Ternes TA, Haberer K, Mehlich A, Ballwanz F, Kratz KL (1998). Determination of antibiotics in different water compartments via liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A* 815: 213-223.
- Holm JV, Ruge K, Bjerg PL, Christensen TH (1995). Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). *Environ. Sci. Technol.* 29:1415-1420.
- Hu XG, Zhou QX, Luo Y (2010). Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, Northern China. *Environ.*

- Pollut. 158(9):2992–2998.
- IDMA (2009). India's quality affordable generics: for global healthcare 47<sup>th</sup> Annual Publication IDMA 2009.
- Jia A, Wan Y, Xiao Y, Hu J (2012). Occurrence and fate of quinolone and fluoroquinolone antibiotics in a municipal sewage treatment plant. *Water Res.* 46:387-394.
- Jjemba PK (2002a). The effect of chloroquine, quinacrine, and metronidazole on both soybean plants and soil microbiota. *Chemosphere* 46:1019-1025.
- Jjemba PK (2002b). The potential impact of veterinary and human therapeutic agents in manure and biosolids on plants grown on arable land: a review. *Agric. Ecosyst. Environ.* 93:267-278.
- Jongbloed AW, Lenis NP (1998). Environmental concerns about animal manure. *J. Anim. Sci.* 76:2641-2648.
- Junker T, Alexy R, Knacker T, Kummerer K (2006). Biodegradability of C-14-labeled antibiotics in a modified laboratory scale sewage treatment plant at environmentally relevant concentrations. *Environ. Sci. Technol.* 40:318-24.
- Karci A, Balcioglu IA (2009). Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. *Sci. Total Environ.* 407:4652-4664.
- Kay P, Blackwell PA, Boxall ABA (2005). Transport of veterinary antibiotics in overland flow following the application of slurry to arable land. *Chemosphere* 59:951-959.
- Kemper N (2008). Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indic.* 8:1-13.
- Kerry J, Coyne R, Gilroy D, Hiney M, Smith P (1996). Spatial distribution of oxytetracycline and elevated frequencies of oxytetracycline resistance in sediments beneath a marine salmon farm following oxytetracycline therapy. *Aquaculture* 145:31-39.
- Kim KR, Owens G, Kwon SI, So KH, Lee DB, Ok YS (2011). Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. *Water Air Soil Pollut.* 214:163–174.
- Kim S, Eichhorn P, Jensen JN, Scott WA, Aga DS (2005). Removal of antibiotics in wastewater: effect of hydraulic and solid retention times on the fate of tetracycline in the activated sludge process. *Environ. Sci. Technol.* 39:5816-5823.
- Kim SC, Carlson K (2006). Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. *Water Res.* 40: 2549–2560.
- Kim SV, Carlson K (2005). LC-MS2 for quantifying trace amounts of pharmaceuticals compounds in soil and sediment matrices. *Trends Anal. Chem.* 24(7):635-644.
- Kim Y, Jung J, Oh SR, Choi K (2008). Aquatic toxicity of cartap and cypermethrin to different life stages of *Daphnia magna* and *Oryzias latipes*. *J. Environ. Sci. Health Part B* 43: 56–64.
- Kinney CA, Furlong ET, Zaugg SD, Burkhardt MD, Werner SL, Cahill JD, et al. (2006a). Survey of organic wastewater contaminants in biosolids destined for land application. *Environ. Sci. Technol.* 40: 7207–7215.
- Kjaer J, Olsen P, Bach K, Barlebo HC, Ingerslev F, Hansen M, Sorensen BH (2007). Leaching of estrogenic hormones from manure-treated structured soils. *Environ. Sci. Technol.* 41: 3911-3917.
- Knapp CW, Dolfing J, Ehlert PAI, Graham DW (2010). Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940. *Environ. Sci. Technol.* 44:580-587.
- Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: a national reconnaissance. *Environ. Sci. Technol.* 36: 1202-1211.
- Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD (2004). Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. *Sci. Total Environ.* 328:119-130.
- Kools SA, Moltmann JF, Knacker T (2008). Estimating the use of veterinary medicines in the European Union. *Regul. Toxicol. Pharmacol.* 50:59–65.
- Kotzerke A, Fulle M, Sharma S, Kleineidam K, Welzl G, Lamshöft M, Schloter M, Wilke B (2011). Alterations in total microbial activity and nitrification rates in soil due to amoxicillin-spiked pig manure. *J. Plant Nutr. Soil Sci.* 174(1):56-64.
- Kotzerke A, Sharma S, Schauss K, Heuer H, Thiele-Bruhn S, Smalla K, Wilke BM, Schloter M (2008). Alterations in soil microbial activity and N-transformation processes due to sulfadiazine loads in pig manure. *Environ. Pollut.* 153(2):315-322.
- Kreuzig R, Höltge S (2005). Investigations on the fate of sulfadiazine in manured soil: laboratory experiments and test plot studies. *Environ. Toxicol. Chem.* 24(4):771-776.
- Kumar K, Gupta SC, Chander Y, Singh AK (2005). Antibiotic use in agriculture and their impact on terrestrial environment. *Adv. Agron.* 87:1-54.
- Kummerer K (2001). Drugs in the environment: emission of drugs, diagnostic aids, and disinfectants into wastewater by hospitals in relation to other sources—a review. *Chemosphere* 45: 957–969.
- Kummerer K (2004). Resistance in the environment. *J. Antimicrob. Chemother.* 54:311-320.
- Kummerer K (2009). Antibiotics in the aquatic environment- a review - part I. *Chemosphere* 75:417-434.
- Kurunthachalam SK (2012). Pharmaceutical substances in India are a point of great concern ? *Hydrol. Curr. Res.* 3:3-5.
- Lancini G, Parenti F (1982). *Antibiotics: an integrated view*. Springer, New York, p. 253.
- LaPara TM, Burch TR, McNamara PJ, Tan DT, Yan M, Eichmiller JJ (2011). Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environ. Sci. Technol.* 45:9543-9549.
- Larsson DG, Fick J (2009). Transparency throughout the production chain—a way to reduce pollution from the manufacturing of pharmaceuticals. *Regul. Toxicol. Pharmacol.* 53 (3): 161-163.
- Larsson DJG, de-Pedro C, Paxeus N (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J. Hazard. Mater.* 148:751-755.
- Le Minh N, Khan SJ, Drewes JE, Stuetz RM (2010). Fate of antibiotics during municipal water recycling treatment processes. *Water Res.* 44:4295-323.
- Li ZJ, Yao ZP, Zhang J, Liang YC (2008). A review on fate and ecological toxicity of veterinary antibiotics in soil environments. *Asian J. Ecotoxicol.* 3(1):15-20.
- Lianfeng Du, Wenke Liu (2012). Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. *Agron. Sustain. Dev.* 32:309-327.
- Lin AYC, Tsai YT (2009). Occurrence of pharmaceuticals in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. *Sci. Total Environ.* 407:3793-3802.
- Lindberg R, Jarnheimer PA, Olsen B, Johansson M, Tysklind M (2004). Determination of antibiotic substances in hospital sewage water using solid-phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere* 57:1479-1488.
- Lindberg RH, Wennberg P, Johansson MI (2005). Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatment plants in Sweden. *Environ. Sci. Technol.* 39:3421-3429.
- Lindsey ME, Meyer TM, Thurman EM (2001). Analysis of trace levels of sulfonamide and tetracycline antimicrobials in groundwater and surface water using solid-phase extraction and liquid chromatography/mass spectrometry. *Anal. Chem.* 73:640-646.
- Liu F, Ying GG, Tao R, Zhao LL, Yang JF, Zhao LF (2009a). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environ. Pollut.* 157:1636-1642.
- Loganathan B, Phillips M, Mowery H, Jones-Lepp T (2009). Contamination profiles and mass loadings of macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant. *Chemosphere* 75:70-77.
- Loo VG, Poirier L, Miller MA, Oughton M, Libman MD, Michaud S, Bourgault AM, Nguyen T, Frenette C, Kelly M, Vibien A, Brassard P, Fenn S, Dewar K, Hudson TJ, Horn R, René P, Monczak Y, Dascal A (2005). A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N. Engl. J. Med.* 353 (23):2442-2449.
- Lugo-Melchor Y, Quinones B, mezquita-Lopez BA, Leon-Felix J, Garcia-Estrada R, Chaidez C (2010). Characterization of tetracycline resistance in *Salmonella enterica* strains recovered from irrigation

- water in the Culiacan valley. Mexico. *Microb. Drug Resist.* 16:185-190.
- Managaki S, Murata A, Takada H, Tuyen BC, Chiem NH (2007). Distribution of macrolides, sulfonamides, and trimethoprim in tropical waters: ubiquitous occurrence of veterinary antibiotics in the Mekong Delta. *Environ. Sci. Technol.* 41(23):8004–8010.
- Martinez-Carballo E, Gonzalez-Barreiro C, Scharf S, Gans O (2007). Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environ. Pollut.* 148 (2):570-579.
- Mathew AG, Cissell R, Liamthong S, Cissell R, Liamthong S (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathog. Dis.* 4 (2):115-33.
- McDonald LC (2005). *Clostridium difficile*: responding to a new threat from an old enemy. *Infect. Control Hosp. Epidemiol.* 26(8):672-675.
- McQuillan D, Hopkins S, Chapman T, Sherrell K, Mills D (2002). Pharmaceutical residues in ambient water: initial surveillance in New Mexico, USA. Presented at 7th annual New Mexico environmental health conference 28-30 October 2002, Albuquerque, New Mexico; 2002.
- Middleton JH, Salierno JD (2012). Antibiotic resistance in triclosan tolerant fecal coliforms isolated from surface waters near wastewater treatment plant outflows (Morris County, NJ, USA). *Ecotoxicol. Environ. Saf.* 1-10.
- Miege C, Choubert JM, Ribeiro L, Eusebe M, Coquery M (2009). Fate of pharmaceuticals and personal care products in wastewater treatment plants—conception of a database and first results. *Environ. Pollut.* 157:1721-1726.
- Migliore L, Brambilla G, Casoria P, Civitareale C, Cozzolino S, Gaudio L (1996). Effect of sulphadimethoxine contamination on barley (*Hordeum distichum* L., Poaceae, Liliopsida). *Agric. Ecosyst. Environ.* 60:121-128.
- Minh TB, Leung HW, Loi IH, Chan WH, So MK, Mao JQ, et al. (2009). Antibiotics in the Hong Kong metropolitan area: ubiquitous distribution and fate in Victoria Harbour. *Mar. Pollut. Bull.* 58:1052-1062.
- Monteiro SC, Boxall ABA (2010). Occurrence and fate of human pharmaceuticals in the environment. *Rev. Environ. Contam. T.* 202:53-154.
- Morris AK, Masterton RG (2002). Antibiotic resistance surveillance: action for international studies. *J. Antimicrob. Chemother.* 49(1):7–10.
- Munoz I, Gómez-Ramos MJ, Agüera A, Fernández-Alba AR, García-Reyes JF, Molina-Díaz A (2009). Chemical evaluation of contaminants in wastewater effluents and the environmental risk of reusing effluents in agriculture. *Trends Analyt. Chem.* 28: 676–694.
- Mutiyaar PK, Mittal AK (2013). Occurrences and fate of an antibiotic amoxicillin in extended aeration-based sewage treatment plant in Delhi, India: a case study of emerging pollutant. *Desalination Water Treat.* doi:10.1080/19443994.2013.770199.
- Mutiyaar PK, Mittal AK (2014). Occurrences and fate of selected human antibiotics in influents and effluents of sewage treatment plant and effluent-receiving river Yamuna in Delhi (India). *Environ. Monit. Assess.* 186:541-557.
- Oetken M, Bachmann J, Schulte-Oehlmann U, Oehlmann J (2004). Evidence for endocrine disruption in invertebrates. *Int. Rev. Cytol.* 236:1-44.
- Onesios KM, Yu JT, Bouwer EJ (2009). Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: A review. *Biodegradation* 20:441-466.
- Parveen S, Lukasik J, Scott TM, Tamplin ML, Portier KM, Sheperd S, Braun K, Farrah SR (2006). Geographical variation in antibiotic resistance profiles of *Escherichia coli* isolated from swine, poultry, beef and dairy cattle farm water retention ponds in Florida. *J. Appl. Microbiol.* 100:50-57.
- Pearson C (2007). Antibiotic Resistance Fast-Growing Problem Worldwide. Voice of America. Archived from the original on 2<sup>nd</sup> December 2008.
- Rabølle M, Spliid NH (2000). Sorption and mobility of metronidazole, olaquinox, oxytetracycline and tylosin in soil. *Chemosphere* 40(7):715-722.
- Ramana S, Biswas AK, Kundu S, Saha JK, Yadava RBR (2002). Effect of distillery effluent on seed germination in some vegetable crops. *Bioresour. Technol.* 82(3): 273-275.
- Reinthal FF, Posch J, Feierl G, Wüst G, Haas D, Ruckebauer G, Mascher F, Marth E (2003). Antibiotic resistance of *E. coli* in sewage and sludge. *Water Res.* 37(8):1685-90.
- Sacher F, Lange FT, Brauch HJ, Blankenhorn I (2001). Pharmaceuticals in groundwaters: analytical methods and results of a monitoring program in Baden-Württemberg, Germany. *J. Chromatogr. A.* 938:99-210.
- Sapkota AR, Curriero FC, Gibson KE, Schwab KJ (2007). Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. *Environ. Health Perspect.* 115:1040-1045.
- Sassman SA, Lee LS (2005). Sorption of three tetracyclines by several soils: role of pH and cation exchange. *Environ. Sci. Technol.* 39:7452-7459.
- Shah SQA, Colquhoun DJ, Nikuli HL, Sorum H (2012). Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. *Environ. Sci. Technol.* 46: 8672-8679.
- Stuer-Lauridsen F, Birkved M, Hansen LP, Holten Lutzhuft HC, Halling-Sørensen B, Halling-Sørensen B (2000). Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. *Chemosphere* 40:783-793.
- Su HC, Ying GG, Tao R, Zhang RQ, Zhao JL, Liu YS (2012). Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in *Escherichia coli* isolated from Dongjiang River, South China. *Environ. Pollut.* 169:42-49.
- Tao R, Ying GG, Su HC, Zhou HW, Sidhu JPS (2010). Detection of antibiotic resistance and tetracycline resistance genes in *Enterobacteriaceae* isolated from the Pearl Rivers in South China. *Environ. Pollut.* 158:2101-2109.
- Ternes TA, Stuber J, Herrmann N, McDowell D, Ried A, Kampmann M, Teiser B (2003). Ozonation: a tool for removal of pharmaceuticals, contrast media, and musk fragrances from wastewater? *Water Res.* 37: 1976-1982.
- Thiele-Bruhn S (2003). Pharmaceutical antibiotic compounds in soils: a review. *J. Plant Nutr. Soil Sci.* 166:45–167.
- Thomashow LS, Weller DM (1995). Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stacey G, Keen N (eds) *Plant-microbe interactions*, vol 1. Chapman & Hall, New York, pp. 187–235.
- LaPara TM, Burch TR, McNamara PJ, Tan DT, Yan M, Eichmiller JJ (2011). Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environ. Sci. Technol.* 45 (22): 9543-9549.
- Topp E, Chapman R, Devers-Lamrani M, Hartmann A, Marti R, Martin-Laurent F, Sabourin L, Scott A, Sumarah M (2013). Accelerated Biodegradation of Veterinary Antibiotics in Agricultural Soil following Long-Term Exposure, and Isolation of a Sulfamethazine-degrading Microbacterium sp. *J. Environ. Qual.* 42 (1):173-178.
- UCS (2001). Union of Concerned Scientist-Hogging it!: estimates of antimicrobial abuse in livestock. [http://www.ucsusa.org/food\\_and\\_environment/antibiotic\\_resistance/indexN.p.4](http://www.ucsusa.org/food_and_environment/antibiotic_resistance/indexN.p.4).
- Underwood JC, Harvey RW, Metge DW, Repert DA, Baumgartner LK, Smith RL, et al. (2011). Effects of the antimicrobial sulfamethoxazole on groundwater bacterial enrichment. *Environ. Sci. Technol.* 45:3096-101.
- USP (2003). *Veterinary Pharmaceutical Information Monographs – Antibiotics Journal of Veterinary Pharmacology and Therapeutics*, 26, supplement watershed. *Water Res.* 40:2549-2560.
- Varga C, Rajic A, McFall ME, Avery BP, Reid-Smith RJ, Deckert A, Checkley SL, McEwen SA (2008). Antimicrobial resistance in generic *Escherichia coli* isolated from swine fecal samples in 90 Alberta finishing farms. *Can. J. Vet. Res. Can. Rech. Vet.* 72:175-180.
- Verlicchi P, Aukidy M Al, Zambello E (2012). Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment-A review. *Sci. Total Environ.* 429: 123-155.
- Wang M, Tang JC (2010). Research of antibiotics pollution in soil environments and its ecological toxicity. *J. Agro-Environ. Sci.* 29(supl): 261-266.
- Watkinson AJ, Murby EJ, Costanzo SD (2007). Removal of antibiotics in

- conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling. *Water Res.* 41:4164-4176.
- Watkinson AJ, Murby EJ, Kolpin DW, Costanzo SD (2009). The occurrence of antibiotics in an urban watershed: from wastewater to drinking water. *Sci. Total Environ.* 407: 2711-2723.
- Watts CD, Craythorne B, Fielding M, Killops SD (1982). Nonvolatile organic compounds in treated waters. *Environ. Health Perspect.* 46: 87-89.
- Wei X, Wu SC, Nie XP, Yediler A, Wong MH (2009). The effects of residual tetracycline on soil enzymatic activities and plant growth. *J. Environ. Sci. Health Part B* 44:461-471.
- WHO (2002). Use of antimicrobials outside human medicine and resultant antimicrobial resistance in humans. World Health Organization. Archived from the original on 13 May 2004.
- Witte W (1998). Medical consequences of antibiotic use in agriculture. *Science* 279:996-997.
- Xu W, Zhang G, Li X, Zou S, Li P, Hu Z, Li J (2007). Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD) South China. *Water Res.* 41: 4526-4534.
- Yang JF, Ying GG, Zhao JL, Tao R, Su HC, Liu YS (2011). Spatial and seasonal distribution of selected antibiotics in surface waters of the Pearl Rivers, China. *J. Environ. Sci. Health Part B* 46:272-280.
- Yang S, Cha J, Carlson K (2005). Simultaneous extraction and analysis of 11 tetracycline and sulfonamide antibiotics in influent and effluent domestic wastewater by solid-phase extraction and liquid chromatography-electrospray ionization tandemmass spectrometry. *J. Chromatogr. A* 1097:40-53.
- Yang YD, Chen DH, Huang MH (2010). The source of antibiotics in the environment and progress of its ecological impact research. *Environ. Sci. Manag.* 35(1):140-143.
- Yao JH, Niu DK, Li ZJ, Liang YC, Zhang SQ (2010). Effects of antibiotics oxytetracycline on soil enzyme activities and microbial biomass in wheat rhizosphere. *Sci. Agric. Sin.* 43 (4):721-728.
- Ye Z, Weinberg HS, Meyer MT (2004). Occurrence of antibiotics in drinking water. In: Proceedings of the 4<sup>th</sup> International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water (CD-ROM), 13-15 October 2004, Minneapolis, MN. p. 138-142. <http://info.ngwa.org/gwof/pdf/042379983.pdf> (accessed on March, 2014).
- Zhang J, Li Z, Ge G, Sun W, Liang Y, Wu L (2009). Impacts of soil organic matter, pH and exogenous copper on sorption behavior of norfloxacin in three soils. *J. Environ. Sci.* 21(5):632-640.
- Zhang R, Zhang G, Zheng Q, Tang J, Chen Y, Xu W, et al. (2012). Occurrence and risks of antibiotics in the Laizhou Bay, China: impacts of river discharge. *Ecotoxicol. Environ. Saf.* 80:208-215.
- Zhang Tong, Li Bing (2011). Occurrence, Transformation, and fate of antibiotics in municipal wastewater treatment plants. *Crit. Rev. Environ. Sci. Technol.* 41(11):951-998.
- Zhou LJ, Ying GG, Liu S, Zhao JL, Yang B, Chen ZF, Lai HJ (2013). Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. *Sci. Total Environ.* 452-453:365-376.
- Zhou LJ, Ying GG, Zhao JL, Yang JF, Wang L, Yang B, et al. (2011). Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China. *Environ. Pollut.* 159:1877-1885.
- Zielezny Y, Groeneweg J, Vereecken H, Tappe W (2006). Impact of sulfadiazine and chlortetracycline on soil bacterial community structure and respiratory activity. *Soil Biol. Biochem.* 38:2372-2380.
- Ziylan A, Ince NH (2011). The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: treatability by conventional and non-conventional processes. *J. Hazard. Mater.* 187:24-37
- Zuccato E, Calamari D, Natangelo M, Fanelli R (2000). Presence of therapeutic drugs in the environment. *Lancet* 355:1789-1790.
- Zuccato E, Castiglioni S, Fanelli R (2005). Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. *J. Hazard. Mater.* 122:205-209.