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Assessment of Antibiotics and Antibiotic Resistant Bacteria in Hospital and Municipal Wastewaters

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The present study was carried out to investigate the occurrence of ciprofloxacin (CIP) and norfloxacin (NOR) in wastewaters of three teaching hospitals of Lahore along with assessment of antibiotic resistant bacteria in the wastewaters. The concentrations of CIP and NOR in the wastewaters of hospitals were ranged from 126.86 to 48.23 μg/L and 50.23 to 7.69 μg/L, respectively. However, the amount of antibiotics present in the municipal wastewater drains before mixing the hospital wastewaters was 2.08 to 1.31 μg/L of CIP and 1.17 to 0.25 μg/L of NOR. In municipal wastewater downstream to the hospitals, concentrations of both antibiotics were increased viz., 2.96 to 1.63 μg/L (CIP), and 1.55 to 0.30 μg/L (NOR). Among the *Escherichia coli* (*E. coli)* isolated from hospital wastewaters, 66% were resistant to CIP and 83% were resistant to NOR. In case of *Salmonella typhimurium* (*S. typhi)*, 83% were resistant to CIP and 66% were resistant to NOR. *E. coli* isolated from municipal upstream to hospital wastewater were 50% resistant to CIP and NOR. Isolates of *S. typhi* were 50% resistant to CIP and 33% resistant to NOR. The results revealed that hospital wastewater are major source of antibiotics and antibiotic resistant bacteria in aquatic environment.

Key words: Hospital wastewater, Ciprofloxacin, Norfloxacin, *Escherichia coli*, *Salmonella typhimurium*.

Occurrence, fate and potential toxic effects of antibiotics have become an important research area during the resent years[1]. The problem of antibiotic resistant bacteria was emerged after the first clinical use of penicillin G during the early 1940s. Efforts were started to overcome the problem by developing new antibiotics. However, soon the bacteria started acquiring resistance to the newly developed antimicrobial agents and started surviving as multi drug resistant pathogens[2,29]. About 40000 antibiotics have so far identified, 80 among them are in use for treatments of different types of bacterial infections. Most of the antibiotics are product of living cells while some like penicillin and cephalosporin are semi synthetic. Because of large variety of antibiotics, they are classified into different groups, and member of each group have properties similar to each other[3].

Antibiotics are very important in modern lifestyle and thousands of tons of antibiotics used per year globally[4]. Most of antibiotics are not completely metabolized in bodies of living organisms; hence excreted from human and animal bodies in active form[5]. When antibiotics transported from humans or farm animals to aquatic environment, they lead to produce antibiotic resistant bacteria, which increase health risks to both humans and animals[6]. Wastewaters from

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hospitals are important source of variety of antibiotics as compared to wastewaters from household activities because of excessive use and less dilution[7]. Other important sources of antibiotics include pharmaceutical processes and disposal of expired antibiotics[5]. These compounds can be detected from aquatic environment at different concentrations ranging from ng L^{-1} to ìg L^{-1}, where these compounds become pseudo-persistent[8]. Antibiotics released from different sources may reach even to drinking water if not removed by any treatment process or decomposed by any environmental factor. Some antibiotics like penicillins and ampicillin are biodegradable in aquatic environment but most of the antibiotics are difficult to remove by ordinary wastewater treatment facilities[9]. Some antibiotics like fluoroquinolones can persist for longer period of time in aquatic environment[10], therefore fluoroquinolones are frequently detected from aquatic environment[11].

Antibiotics present in water bodies are indirectly toxic to human, farm animals and aquatic life as they produce resistance in bacteria and algae at very little concentration. Hence, antibiotics have the tendency to upset basic cycles including nitrification and denitrification which are essential to aquatic ecosystem, agriculture and animal production[12,13].

Present study was carried out to address two main issues;

To investigate the presence of two important fluoroquinolone antibacterial agents (CIP and NOR) from hospital effluents and also from municipal wastewater drains of Lahore receiving the hospitals wastewater.

To explore the persistence of antibiotic-resistance-bacteria from wastewaters of selected sites.

MATERIALS AND METHODS

Water samples and analysis

Hospital wastewater samples were collected from three teaching hospitals (Mayo, Services and Jinnah) of Lahore. Composite samples were collected from raw hospital wastewater effluents and from the municipal wastewater drains before and after mixing the hospital wastewaters. To investigate temporal variations in the concentration of the selected antibiotics in the raw wastewaters of hospitals, the samples were collected during four different times i.e. 7-11, 11-15, 15-19 and 19-23 hrs. Quantity of each sample was 4 L and were collected in amber glass bottles and transfer to laboratory in ice box and saved with Na_2EDTA at 4 ^{0}C[6].

The raw wastewater samples were first filtered through 0.45µm membrane filter and acidify by adding H_2SO_4 up to pH 3[4]. Extraction was carried out by Solid Phase Extraction (SPE); the cartridges were first rinsed with 2 ml of methanol followed by 2 ml of water. The required compounds were eluted from the cartridges by using 2 ml of methanol. The extracted samples were analyzed by high performance liquid chromatography (LC-20A Shimadzu) equipped with UV-visible and photodiode array detector. The analytical column (RP-18e 250mm×4.6mm, 5µm manufactured by Merck) at room temperature was used for analysis[4,14].

Bacterial sampling and analysis

Sterilized amber glass bottles were used for each sample and capacity of each bottle was 4L. Some portion of the bottles was kept empty for shaking of samples. The samples were transfer to laboratory in ice box. Identification and isolation of *S. typhi* was done by selective growth media Brilliance™ Salmonella Agar (Oxoid) by incubating at 37 ^{0}C for 24 hours. Purple colonies were selected as *S. typhi*. E. coli was identified and isolated by *Brilliance™ E. coli/coliform Selective Agar (Oxoid) by incubating at 37* ^{0}C for 24 hours. Purple color colonies were regarded as *E. coli*. Both *S. typhi* and *E. coli* were subcultured on the nutrient agar and then kept at 4 ^{0}C for further examination[15,16]. Bacteria were isolated from the sites with higher concentrations of the two antibiotics, because sites with higher concentrations considered to having more resistant strains as compare to sites with less concentration[17].

To observe the resistance level of *E. coli* and *S. typhi*, different concentrations of the antimicrobial agents were prepared by using Oxoid Iso-sensitest broth, the pathogens were incubated at 37 ^{0}C for 24 hours and monitor the growth of the bacteria (as turbidity) by measuring optical density at 600 nm. Duplicate samples of each antibiotic concentration were grown. Haemocytometer was used to prepare same size (10^3 CFU/ml) of inoculums[18,19,20].

RESULTS AND DISCUSSION

Table 1 shows the temporal variations of CIP and NOR in wastewater samples collected from the three teaching hospitals of Lahore and the concentrations of the antibiotics in the municipal wastewater both before and after mixing the untreated hospital wastewater. CIP was more abundant as compared to NOR in untreated hospital wastewater ranged from 120.67 to 48.23 µg/L and 50.23 to 7.69 µg/L, respectively. Maximum concentration of CIP and NOR in the untreated hospital wastewater was noted between 11:00 to 15:00. Concentrations of CIP and NOR in wastewater drains receiving untreated hospital wastewater after mixing, ranged from 2.96 to 1.63 µg/L and 1.55 to 0.30 µg/L, respectively. While concentration of CIP in municipal wastewater drain before mixing the hospital wastewater ranged from 2.08 to 1.31 µg/L and NOR ranged from 1.17 to 0.25 µg/L.

The values of present study were higher than values of CIP and NOR concentrations 1.1 to

Table 1. Antibiotic concentration in wastewaters of hospitals and receiving municipal wastewater drains of Lahore

Antibiotic	Hospital	Concentrations in (µg/L) Time of sampling (hrs)				Municipal wastewater drain 17 hrs composite sampling	
		07:00 to 11:00	11:00 to 15:00	15:00 to 19:00	19:00 to 23:00	Before mixing	After Mixing
CIP	Mayo	120.67a ±3.24	131.51a ±4.31	79.27a ±6.46	126.86a ±4.16	2.08a ±0.05	2.96a ±0.22
	Services	89.77c ±3.5	106.58c ±5.14	71.65b ±2.43	94.61b ±4.61	1.68b ±0.14	2.21b ±0.12
	Jinnah	94.25b ±6.7	113.0b ±3.53	48.23c ±4.05	90.33c ±6.72	1.31c ±0.15	1.63c ±0.41
NOR	Mayo	38.50a ±2.43	50.23a ±6.07	30.81a ±2.71	41.92a ±1.32	1.17a ±0.11	1.55a ±0.30
	Services	26.02b ±3.1	41.54b ±4.11	19.43b ±2.18	34.20b ±3.24	0.61b ±0.07	0.74b ±0.09
	Jinnah	11.47c ±0.91	19.34c ±0.46	7.69c ±1.27	17.18c ±0.37	0.25c ±0.04	0.30c ±0.01

Treatment means followed by different letters in each column for both ciprofloxacin and norfloxacin are significantly different at p=0.05 according to Duncan's Multiple Range Test.

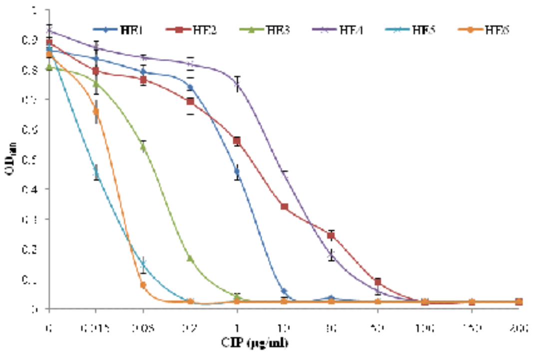

Fig. 1. Effect of CIP on cell growth of *E.coli* collected from hospital wastewater

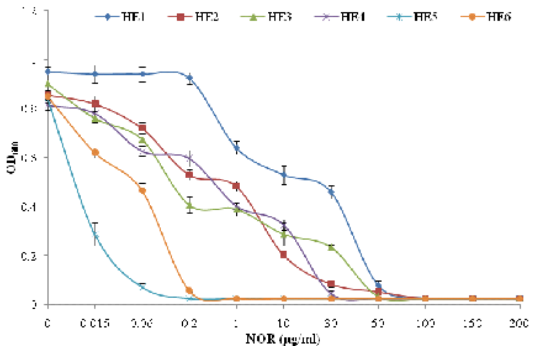

Fig. 2. Effect of NOR on cell growth of *E.coli* collected from hospital wastewater

44 and 0.9 to 17 µg/L, respectively reported in Vietnam[7] and values measured in hospital wastewaters in Switzerland viz., 0.3–29 µg/L for CIP, 0.3–8 µg/L for NOR[21]. Concentrations of antibiotics which were comparable to the present study were however measured in Sweden[4] (3.6–101 µg/L for CIP) and in Germany 0.7–125 µg/L for CIP[23], and 44 µg/L for NOR[22]. The concentration of CIP in wastewaters effluents was observed up to 3.6 to 101 µg/L, and temporal variation in the concentration of CIP and some other antibiotics was also observed in the hospital wastewaters[4].

Antibiotics found in effluents of wastewaters treatment plant (WWTP) were up to 64µg/L; dominant groups were â-lactam, fluoroquinolone and sulphonamide. The WWTP installed for the treatment of hospital wastewater was removing antibiotic up to 80%. Prominent groups of antibiotics found in effluent of the WWTP were fluoroquinolone, macrolide and sulphonamide. Specially designed WWTP for the removal of antibiotics would be installed for the removal of antibiotics from hospital wastewater[12]. The concentration of CIP and NOR in the hospital wastewater depends on many factors like specialization of a hospital, number of occupied beds, time of administration and prescription habits and availability of the antibiotics[7].

E. coli and *S. typhi* isolated from wastewaters of Mayo hospital, Lahore and municipal wastewaters (MWW) drain before mixing the hospital wastewater were selected for antibiotic resistance tests and to compare the resistance level between hospital and MWW drain bacteria. Bacterial isolates were considered resistant if survive above MIC$_{90}$ values. Among the 6 isolates of *E. coli* from the hospital wastewater, 4 (66%) and 5 (83%) were resistant to CIP and NOR, respectively as shown in the Fig. 1 and 2. While the 6 isolates of *E. coli* collected from MWW drain before mixing the hospital wastewater, 3 (50%) and 3 (50%) were resistant to

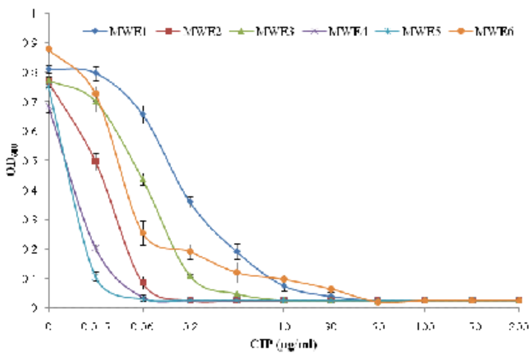

Fig. 3. Effect of CIP on cell growth of *E.coli* collected from MWW drain of Myo hospital

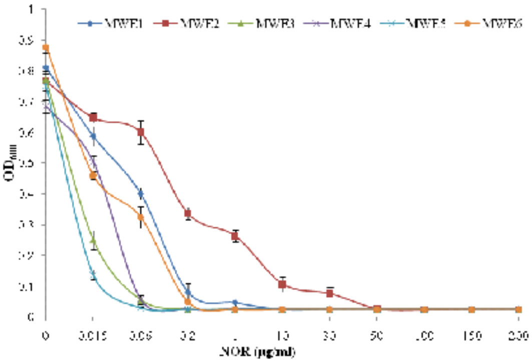

Fig. 4. Effect of NOR on cell growth of *E.coli* collected from MWW drain of Mayo hospital

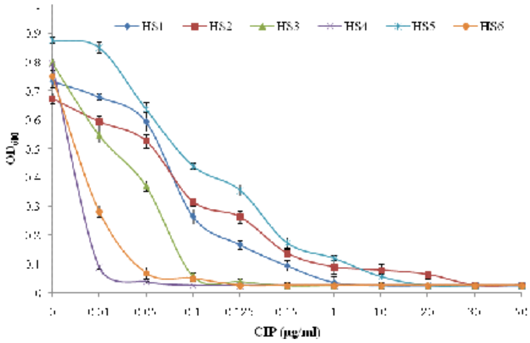

Fig. 5. Effect of ciprofloxacin on cell growth of *S. typhi* collected from hospital wastewater

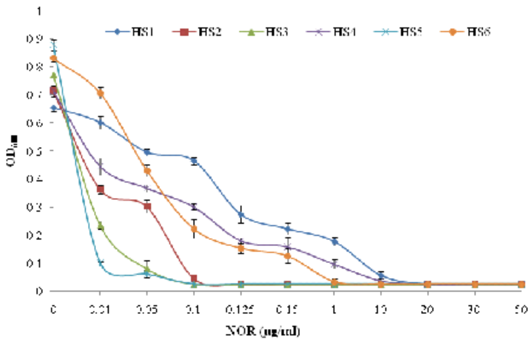

Fig. 6. Effect of NOR on cell growth of S. typhi collected from hospital wastewater

CIP and NOR respectively as can be seen in Fig. 3 and 4. In case of *S. typhi*, isolated from the hospital wastewater, 5 (83%) and 4 (66%) were resistant to CIP and NOR respectively (Fig. 5 and 6). While the *S. typhi* isolated from MWW drain before mixing the hospital wastewater, 3 (50%) resistant to CIP and 2 (33%) were resistant to NOR (Fig. 7 and 8). It was recorded that hospital isolates were able to survive at higher concentrations as compare to MWW drain isolates.

It was reported that 50% isolates were resistant to CIP[24], while 53% resistant *E. coli* for both CIP and NOR was also reported[7]. A value of 55% resistant *E. coli* for CIP was reported by a previous study[25]. About 238 bacteria isolated from drinking water bottles of different brands and studied for antibiotic resistance. It was noted that all the isolated bacteria were resistant to at least three antibiotics[26]. Isolates from clinical wastewater showed high resistant to antibiotics than the wastewater isolates. Out of 27 isolates from sewage, 38% were found resistant to aztreonam while 50% of clinical isolates were resistant to the antibiotic. Hence, it is clear that sewage wastewater which receiving untreated hospital wastewater found to be a major source of multidrug resistance bacteria to environment[27]. Different species of bacteria were collected from samples of hospital wastewaters, activated sludge, municipal wastewaters and rivers receiving the wastewaters. It was demonstrated that among all the environmental samples, hospital isolates were more antibiotic resistant as compare to others[28]. That is the reason that many compounds are not as active against bacteria as they were[29,30].

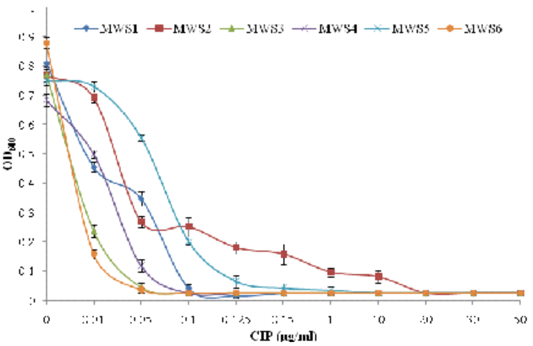

Fig. 7. Effect of CIP on cell growth of *S. typhi* collected from MWW drain of Mayo hospital

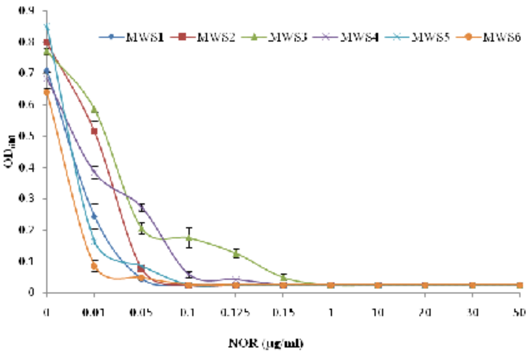

Fig. 8. Effect of NOR on cell growth of *S. typhi* collected from MWW drain of Mayo hospital

CONCLUSIONS

The present study provided information on occurrence and temporal variations of two antimicrobial agents (CIP and NOR) of fluoroquinolone group found in the untreated wastewaters of three teaching hospitals of Lahore. The concentrations of two antibiotics under study were comparable as well as higher to the concentrations investigated in previous published studies in Europe and USA. It is evident now that hospitals are the major contributors in channelizing antibiotics and antibiotic resistant bacteria to the aquatic environment. Hospitals must be equipped with WWTPs especially designed for the antibiotic removal because different studies revealed that ordinary wastewater treatment plants are not able to completely remove antibiotics from hospital wastewater. In summary, wastewaters from various sources ultimately enters into the water bodies (river/canals/streams etc.), and is primarily used for irrigation[31] or consumption of livestock; it can cause serious health problems due to the presence of multidrug resistant bacteria in it.

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