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Profiling of meropenem-resistant bacteria in a river receiving wastewater effluent from a pharmaceutical industrial unit

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The aim of the present study was to understand the seasonal occurrence and diversity of species of meropenem-resistant bacteria in the Gumuncheon river receiving effluents from a pharmaceutical industry in Seoul, Korea. Water samples were collected from the Gumuncheon river in Kyoung-gi province during winter (January), spring (April), summer (August), and fall (November) of 2018. Water samples were plated in triplicate on tryptic soy agar plates containing 16 mg/L meropenem. Meropenem-resistant bacteria were isolated and genetically identified using 16S rRNA analysis. The predominant bacterial genera identified were Elizabethkingia, Pseudomonas, Chryseobacterium and Stenotrophomonas. Among these; Pseudomonas species Pseudomonas chenguensis and Pseudomonas taiwanesis showed resistance against 15 antibiotics. To prevent the occurrence and spread of meropenem-resistant bacteria in rivers, it is necessary to implement methods that can simultaneously kill multi-drug resistant bacteria and remove antibiotics from pharmaceutical industry effluent discharge. Further, to stop the spread of meropenem-resistant bacteria in environment, effluent discharge water should be stringently assessed for their risk of being an environmental hazard.

Key words: Carbapenem, Meropenem, Multi-drug resistant, Elizabethkingia, Pseudomonas, Chryseobacterium, Stenotrophomonas.

INTRODUCTION

Carbapenem antibiotics, such as meropenem, imipenem, and doripenem, are the last-line of antibiotics used on bacteria resistant to β-lactam antibiotics. In vitro experiments have shown that carbapenem antibiotics have broader antibacterial activities than combinations of penicillin, cephalosporin, and β-lactam/β-lactamase inhibitors (Bassetti et al., 2009). Generally, imipenem, panipenem, and doripenem are effective antibiotics against Gram-positive bacteria (GPB), whereas carbapenem antibiotics, such as meropenem, biapenem,
and ertapenem, are more effective against Gram-negative bacteria (GNB) (Bassetti et al., 2009).

Carbapenem antibiotics enter GNB through outer membrane proteins (OMPs) known as porins (Martinez-Marinez, 2008). Carbapenem antibiotics pass through the periplasmic space and subsequently inhibit peptide cross-linking by permanent acylation of penicillin-binding proteins (PBPs). PBPs facilitate the synthesis of peptidoglycans present in the bacterial cell wall (Hashizume et al., 1984). Cell wall synthesis is a dynamic three-dimensional process with synthesis and autolysis occurring simultaneously. As a result, inhibition of PBPs causes weakening of peptidoglycans, thereby resulting in cell rupture due to osmotic pressure (Van Dam et al., 2009).

Carbapenem resistance has been reported in many bacteria. For example, Enterobacteriaceae including Escherichia coli, Klebsiella spp., Klebsiella pneumoniae, and Enterobacter spp., and GNB, including Stenotrophomonas spp., Streptococcus spp., Staphylococcus spp., Bacillus subtilis, and Bacillus licheniformis show gradual resistance to carbapenem antibiotics often used in clinical practice (Papp-Wallace et al., 2011; Go et al., 2017; Hwang and Kim, 2018). In particular, carbapenem-resistant Enterobacteriaceae (CRE) are becoming important public health risks (Gupta et al., 2011).

The pharmaceutical industry produces effluent discharge which carries various types of antibiotics that are not degraded during the wastewater treatment process. Antibiotics that are discharged without being completely degraded can cause disturbances in aquatic ecosystems and will be hazardous for human life (Kim and Kim, 2016). Pharmaceutical industry wastewater treatment plants may also discharge wastewater directly into rivers if they satisfy the effluent standards for general metrics such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids (SS), total nitrogen (T-N), and total phosphorus (T-P), similar to non-pharmaceutical wastewater treatment plants (Hwang and Kim, 2018).

The purpose of this study was to identify the seasonal frequency of occurrence, species variety, and antibiotic resistance spectrum of meropenem-resistant bacteria in rivers receiving wastewater discharged from a pharmaceutical industrial park.

**MATERIALS AND METHODS**

**Collection area and methods**

Gumuncheon river: from where the study samples were collected, is a tributary of the Balancheon river. The Hyangnam Pharmaceutical Industrial Park, located in Hwaseong-si (Gyeonggi Province, South Korea), houses 10 pharmaceutical companies that discharge wastewater into the Gumuncheon river. We sampled 1L of river water at 37.097598N and 126.902025E in January, April, August, and November of 2018. Samples were collected in sterile collection bottles and refrigerated during transportation to the laboratory (Hwang et al., 2018).

Water temperature, dissolved oxygen (DO), and the pH of the river water were measured using a DO/pH meter (DH-32P, Toa, Japan). Prior to analyzing T-N, T-P, and total organic carbon (TOC), a 0.45 μm pore filter (Advnatec, Japan) was used to remove any suspended solids and temperature, DO, and pH were measured at the sampling point. T-N and T-P were measured using Integral Futura continuous flow analyzer (Shimadzu, Japan), while TOC was measured using TOC-L (Shimadzu, Japan). BOD and COD were analyzed in accordance with the water pollution standard method (NIER, 2018).

**Isolation of heterotrophic bacteria**

To measure the heterotrophic bacterial count within each sample, the samples were diluted 100-fold using sterilized saline solution and 1.0 ml from each dilution each was spread 3 times on 3 agar plates (pour plate agar, Difco, USA) containing 16 mg/L of meropenem (Daewoong Pharmaceutical, Korea) and cultured at 35°C for 48 h. Meropenem-resistant bacteria were subjected to 16S rRNA analysis for species identification. Colony polymerase chain reaction (PCR) was used to amplify the 16S rRNA gene of resistant bacteria from pure colonies (Kim and Kim, 2016). To determine the antibiotic sensitivities of the identified bacteria, disk diffusion assays were performed with ampicillin, cefotizoxime, vancomycin, imipenem, clindamycin, gentamicin, erythromycin, ciprofloxacin, nitrofurans, rifampin, ampicillin/ sulbacatam, aztreonam, spectinomycin, trimethoprim, tetracycline, and chloramphenicol. The diameter of the inhibition zone was measured in mm and interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013; Hwang et al., 2018).

**RESULTS**

The general quality metrics of the river samples are shown in Table 1. Temperature (7.5-28.5°C), pH (7.15-7.45), DO (6.1-11.0 mg/L), BOD (2.7-5.8 mg/L), COD (8.2-15.1 mg/L), T-P 0.103-0.182 mg/L), T-N (8.056-12.566 mg/L), and TOC (5.3-9.2 mg/L) were measured.

The numbers of heterotrophic and meropenem-resistant bacteria identified are shown in Table 2. The counts of heterotrophic bacteria in the winter, spring, summer, and fall seasons were 3.4, 4.0, 5.6 and 5.1 ×10⁴ CFU/ml respectively, while the numbers of meropenem-resistant bacteria were 14.0, 8.3, 1.2 and 9.0 ×10² CFU/ml respectively.

The prevalence of each strain by season is shown in
Table 1. Quality of water sampled from the river receiving pharmaceutical industry discharge water.

| Indicators                   | January          | April            | August           | November         |
|------------------------------|------------------|------------------|------------------|------------------|
| Temperature(°C)              | 7.5              | 20.1             | 28.5             | 18.2             |
| pH                           | 7.42             | 7.15             | 7.34             | 7.45             |
| DO (mg/L)                    | 11.0             | 10.0             | 6.1              | 8.4              |
| BOD (mg/L)                   | 5.8              | 4.0              | 2.7              | 4.3              |
| COD (mg/L)                   | 15.1             | 10.7             | 9.7              | 8.2              |
| Total phosphorus (mg/L)      | 0.145            | 0.127            | 0.182            | 0.103            |
| Total nitrogen (mg/L)        | 12.566           | 8.305            | 9.644            | 8.056            |
| TOC (mg/L)                   | 9.2              | 5.6              | 5.3              | 5.8              |

DO: dissolved oxygen, BOD: biological oxygen demand, COD: chemical oxygen demand, TOC: total organic carbon.

Table 2. Season-wise distribution of culturable bacteria and meropenem resistant bacteria.

| Indicators                              | January            | April             | August            | November          |
|-----------------------------------------|--------------------|-------------------|-------------------|-------------------|
| Heterotrophic bacteria(CFU/ml)          | 3.4×10⁴ ±1.3×10³   | 4.8×10⁴ ±2.6×10³  | 5.6×10⁴ ±1.5×10³  | 5.1×10⁴ ±2.8×10³  |
| Meropenem resistant Bacteria (CFU/ml)    | 4.0×10⁴ ±2.0×10³   | 8.3×10⁴ ±2.1×10³  | 1.2×10⁵ ±4.0×10³  | 9.0×10⁵ ±1.2×10⁵  |
| Meropenem resistant bacteria (%)         | 1.18               | 1.73              | 2.14              | 1.82              |

Table 3. The *Elizabethkingia* genus was the dominant genus in the winter season, and was also partially identified in the spring and fall when the water temperature was low; however, it was not identified in summer. The *Pseudomonas* genus was not identified in the winter and summer but was identified during the spring and the fall. The *Chryseobacterium* genus was identified as the dominant genus in the spring, summer, and winter; the *Stenotrophomonas* genus was also identified in the same periods. The *Cupriavidus*, *Acinetobacter*, and *Pandoraea* genera were identified in the summer season only.

The results of the antibiotic resistance tests of the isolated meropenem-resistant bacteria are shown in Tables 4, 5, and 6. The *Elizabethkingia* genus commonly showed resistance to eight antibiotics (ampicillin, ceftizoxime, vancomycin, imipenem, meropenem, clindamycin, gentamicin, and aztreonam). The *S. maltophilia*, which appeared in the spring, summer, and fall, showed resistance to ten antibiotics (ampicillin, ceftizoxime, vancomycin, imipenem, meropenem, clindamycin, ampicillin/sulbactam, spectinomycin, aztreonam, and trimethoprim). Among bacteria that appeared only in the summer season, *C. plantarum* showed resistance to seven antibiotics (ampicillin, vancomycin, imipenem, meropenem, clindamycin, gentamicin, and aztreonam), *A. junii* showed resistance to nine antibiotics (ampicillin, ceftizoxime, vancomycin, imipenem, meropenem, clindamycin, nitrofurantoin, rifampin, and trimethoprim), and *P. promenusa* showed resistance to eleven antibiotics (colistin, ampicillin, vancomycin, meropenem, clindamycin, gentamicin, erythromycin, nitrofurantoin, rifampin, spectinomycin, and aztreonam).

**DISCUSSION**

We evaluated the occurrence and season-wise prevalence of meropenem-resistant bacteria in Gumuncheon river receiving wastewater effluents from a pharmaceutical industrial unit. The average water temperature during the winter was 7.5°C, which was lower than the temperature of other sessions for the river.
Table 3. Number of meropenem-resistant bacteria detected by season.

| Species                        | Sampling months in 2018 | Total |
|--------------------------------|-------------------------|-------|
|                                | January | April | August | November |       |
| Elizabethkingia amricola       | 11      | -     | -      | 3        | 14    |
| Elizabethkingia ameningoseptica| 1       | -     | -      | -        | 1     |
| Elizabethkingia anophelis      | -       | 1     | -      | -        | 1     |
| Pseudomonas taiwanesus         | -       | 3     | -      | -        | 3     |
| Chryseobacterium indologenes   | -       | 17    | -      | -        | 17    |
| Pseudomonas chengduensis       | -       | 2     | -      | 2        | 4     |
| Stenotrophomonas maltophilia   | -       | 2     | 5      | 4        | 11    |
| Chryseobacterium cucumeris     | -       | -     | 22     | 15       | 37    |
| Cupriavidus plantarum          | -       | -     | 3      | -        | 3     |
| Acinetobacter junii            | -       | -     | 3      | -        | 3     |
| Pandoraea pnomenusa            | -       | -     | 3      | -        | 3     |
| Pseudomonas pseudoalcaligenes   | -       | -     | -      | 3        | 3     |
| **Total**                      | 12      | 25    | 36     | 27       | 100   |

Table 4. Antibiotic resistance spectrum of meropenem-resistant Elizabethkingia miricola, Elizabethkingia meningoseptica, Elizabethkingia anophelis, and Pseudomonas taiwanesis.

| Spectrum          | E. miricola (n=14) | E. meningoseptica (n=1) | E. anophelis (n=1) | P. tawnensis (n=3) |
|-------------------|--------------------|-------------------------|--------------------|--------------------|
|                   | R%                 | R%                      | R%                 | R%                 |
| Ampicillin        | 100                | 100                     | 100                | 100                |
| Ceftizoxime       | 100                | 100                     | 100                | 100                |
| Vancomycin        | 21                 | 100                     | 100                | 100                |
| Imipenem          | 100                | 100                     | 100                | 100                |
| Meropenem         | 100                | 100                     | 100                | 100                |
| Clindamycin       | 57                 | 0                       | 0                  | 100                |
| Gentamicin        | 100                | 0                       | 100                | 33                 |
| Erythromycin      | 79                 | 0                       | 100                | 100                |
| Ciprofloxacin     | 57                 | 0                       | 100                | 100                |
| Nitrofurantoin    | 100                | 100                     | 100                | 100                |
| Rifampin          | 0                  | 0                       | 0                  | 100                |
| Ampicillin/Sulbactam | 100            | 0                       | 100                | 100                |
| Aztreonam         | 100                | 100                     | 100                | 100                |
| Spectinomycin     | 43                 | 0                       | 100                | 100                |
| Trimethoprim      | 57                 | 100                     | 100                | 100                |
| Tetracycline      | 0                  | 0                       | 0                  | 100                |
| Chloramphenicol   | 0                  | 0                       | 0                  | 100                |

The increase in water temperature was assumed to be the result of an inflow of effluents from the sewage treatment plant. The concentration of T-P, a limiting nutrient for microbial growth on water surface, was found to be 0.103-0.182 mg/L, which was exceedingly higher than the concentration needed for microbial growth (100 μg T-P/L). It showed that the sampled river water satisfied the conditions for growth of antibiotic resistant bacteria (Correll, 1999). Our results clearly show that the present allowed limit of T-P concentration (4 mg/L) for pharmaceutical industrial wastewater treatment plant effluents should be lowered, considering the additional
Table 5. Antibiotic resistance spectrum of meropenem-resistant *Chryseobacterium indologenes, Pseudomonas chengduensis, S. maltophilia* and *Chryseobacterium cucumeris*.

| Spectrum       | *C. indologenes* R% (n=17) | *P. chengduensis* R% (n=4) | *S. maltophilia* R% (n=11) | *C. cucumeris* R% (n=37) |
|----------------|-----------------------------|-----------------------------|-----------------------------|--------------------------|
| Ampicillin     | 100                         | 100                         | 100                         | 100                      |
| Ceftizoxime    | 100                         | 100                         | 100                         | 100                      |
| Vancomycin     | 100                         | 100                         | 100                         | 46                       |
| Imipenem       | 6                           | 100                         | 100                         | 0                        |
| Meropenem      | 100                         | 100                         | 100                         | 100                      |
| Clindamycin    | 0                           | 100                         | 100                         | 0                        |
| Gentamicin     | 94                          | 100                         | 55                          | 100                      |
| Erythromycin   | 0                           | 100                         | 73                          | 0                        |
| Ciprofloxacin  | 0                           | 100                         | 0                           | 0                        |
| Nitrofurantoin | 77                          | 100                         | 82                          | 19                       |
| Rifampin       | 0                           | 100                         | 82                          | 0                        |
| Ampicillin/Sulbactam | 100                 | 100                     | 100                         | 100                      |
| Aztreonam      | 100                         | 0                           | 100                         | 100                      |
| Spectinomycin  | 0                           | 100                         | 100                         | 0                        |
| Trimethoprim   | 0                           | 100                         | 100                         | 0                        |
| Tetracycline   | 0                           | 100                         | 0                           | 0                        |
| Chloramphenicol| 0                           | 100                         | 9                           | 0                        |

Table 6. Antibiotic resistance spectrum of meropenem-resistant *Chryseobacterium plantarum, A. junii, Pseudomonas pnomenusa*, and *Pseudomonas pseudoalcaligenes*.

| Spectrum       | *C. plantarum* R% (n=3) | *A. junii* R% (n=3) | *P. pnomenusa* R% (n=3) | *P. pseudoalcaligenes* R% (n=3) |
|----------------|--------------------------|---------------------|--------------------------|-------------------------------|
| Ampicillin     | 100                      | 100                 | 100                      | 100                           |
| Ceftizoxime    | 0                        | 100                 | 0                        | 100                           |
| Vancomycin     | 100                      | 100                 | 100                      | 100                           |
| Imipenem       | 100                      | 100                 | 0                        | 100                           |
| Meropenem      | 100                      | 100                 | 100                      | 100                           |
| Clindamycin    | 100                      | 100                 | 100                      | 100                           |
| Gentamicin     | 100                      | 0                   | 100                      | 0                             |
| Erythromycin   | 0                        | 0                   | 100                      | 100                           |
| Ciprofloxacin  | 0                        | 0                   | 0                        | 0                             |
| Nitrofurantoin | 33                       | 100                 | 100                      | 100                           |
| Rifampin       | 33                       | 100                 | 100                      | 67                            |
| Ampicillin/Sulbactam | 0                  | 0                   | 0                        | 100                           |
| Aztreonam      | 100                      | 0                   | 100                      | 100                           |
| Spectinomycin  | 67                       | 0                   | 100                      | 0                             |
| Trimethoprim   | 0                        | 100                 | 33                       | 100                           |
| Tetracycline   | 0                        | 0                   | 0                        | 0                             |
| Chloramphenicol| 0                        | 33                  | 0                        | 100                           |
amount of T-P that could be introduced into the river from nearby farmland or domestic sewage (Hwang and Kim, 2018).

The percentage of meropenem-resistant bacteria among the heterotrophic bacteria identified in the river samples was 1.18-2.14%, which was higher than the percentage measured in a similar river which did not receive the wastewater effluents (Kim and Kim, 2015). It is known that as the number of non-pathogenic bacteria increases, the number of antibiotic-resistant bacteria could also increase through gene transfer from pathogenic to non-pathogenic bacteria (Levy and Marshall, 2004).

The Elizabethkingia genus contains Gram-negative, obligate aerobic bacillus species, and is an emerging healthcare threat as it has been reported to be associated with various life-threatening infections including sepsis, neonatal meningitis, and nosocomial pneumonia. Moreover, improperly processed animal-derived food and companion animals are known reservoirs for this antibiotic resistant bacterial pathogen. Elizabethkingia anophelis isolated in clinical practice (Figueroa Castro et al., 2017; Lee et al., 2021) and E. anophelis isolated from horses (Johnson et al., 2018) were reportedly susceptible to ciprofloxacin; however, the E. anophelis isolated in the present study was ciprofloxacin-resistant. Further studies are required to determine whether this difference was due to mutation of one or more genes involved in bacterial DNA separation (Drlica and Zhao, 1997). Similarly, E. meningoseptica isolated from clinical samples has shown resistance to various β-lactams and colistin, but is reported to be susceptible to vancomycin (Ratnamani and Rao, 2013). However, the E. meningoseptica isolated in the present study was resistant to not only ampicillin, imipenem, meropenem, and the β-lactam/β-lactamase inhibitor ampicillin/Sulbactam, but also to colistin and vancomycin. On the other hand, the E. meningoseptica isolated in the present study showed greater susceptibility to tetracycline, clindamycin, erythromycin, and gentamicin than E. meningoseptica from hospital effluents described previously. Thus, more in-depth studies are required to determine whether this difference could be attributed to the difference in their source (NIER, 2013; Gullberg et al., 2011). E. miricola, which is an opportunistic oral pathogen, has exhibited resistance to many antibiotics, including imipenem, meropenem, carbapenem, colistin, and gentamicin (Zdziarski et al., 2017; Howard et al., 2020); the E. miricola isolated in the present study also showed resistance to those antibiotics.

There have been no reports of Pseudomonas taiwanesis, a known pathogen of soil microorganisms (Wang et al., 2010) and insects (Chen et al., 2014), acting as a clinical pathogen. However, the P. taiwanesis isolated in the present study showed resistance to sixteen antibiotics (ampicillin, ceftriaxone, vancomycin, imipenem, meropenem, clindamycin, erythromycin, ciprofloxacin, nitrofurantoin, rifampin, ampicillin/sulbactam, spectinomycin, aztreonam, trimethoprim, tetracycline, and chloramphenicol). Similarly, Pseudomonas chenguensis has been reported in landfill leachate (Tao et al., 2014), but has not been identified in clinical samples. The P. chenguensis isolated in the present study showed resistance to ampicillin, ceftriaxone, vancomycin, imipenem, meropenem, clindamycin, gentamicin, erythromycin, ciprofloxacin, nitrofurantoin, rifampin, ampicillin/sulbactam, spectinomycin, trimethoprim, tetracycline, and chloramphenicol. Pseudomonas pseudealcaligenes, another member of the Pseudomonas genus, showed resistance to six antibiotics (ampicillin, ceftriaxone, vancomycin, clindamycin, erythromycin, and chloramphenicol), similar to P. pseudealcaligenes strains isolated from hospital effluents.

Chryseobacterium, which was the dominant genus identified in sampled water during spring, summer, and fall, is a Gram-negative bacillus commonly found in environment. Chryseobacterium indolgenes is an opportunistic pathogen that causes sepsis (McKew, 2014; Izaguirre-Anariba and Sivapalan, 2020). C. indolgenes isolated from patients has been reported to be resistant to ampicillin, amoxicillin-clavulanate, cefepime, meropenem, gentamicin, and nitrofurantoin; however the C. indolgenes isolated in the present study showed resistance to colistin, ampicillin, ampicillin-sulbactam, ceftriaxone, vancomycin, meropenem, gentamicin, and aztreonam. Chryseobacterium cucumeris, another member of the Chryseobacterium genus, was resistant to colistin, ampicillin, ampicillin-sulbactam, ceftriaxone, meropenem, gentamicin, and aztreonam.

Stenotrophomonas maltophilia, isolated in the spring, summer, and fall, is strongly associated with human respiratory infection. It is a multi-drug resistant bacterium commonly found in environment. Due to its low permeability, it is known to be resistant to cephem antibiotics, such as cefepime and ceftazidime, as well as to β-lactams. Moreover, presence of genes encoding β-lactamases, multi-drug resistant efflux pumps, and antibiotic-modifying enzymes confers it with resistance to various antibiotics (Brook, 2012; Adegoka et al., 2017). Similarly, the S. maltophilia isolated in the present study showed resistance to not only β-lactam and cephem antibiotics, such as ampicillin and ceftriaxone, but also to vancomycin, imipenem, meropenem, clindamycin, ampicillin/sulbactam, spectinomycin, aztreonam, and trimethoprim.

Cupriavidus plantarum has been isolated from the plant rhizosphere (Estrada-de Los Santos et al., 2014), but there are no known reports of this bacteria in clinical samples. The C. plantarum identified in the present study
showed resistance to ampicillin, vancomycin, imipenem, meropenem, clindamycin, gentamicin, and aztreonam.

*Acinetobacter junii* is an opportunistic pathogen that has been reported to show resistance to ampicillin and ciprofloxacin (Cayô et al., 2011); in the present study, the *A. junii* showed resistance to a greater number of antibiotics (ampicillin, cefotizoxide, vancomycin, imipenem, meropenem, clindamycin, nitrofurantoin, rifampin, and trimethoprim). Similarly, *Pandoraea pmonenusa* isolated from patients with cystic fibrosis has been reported to show resistance to cephem antibiotics such as cefotizoxide and β-lactam/β-lactamase inhibitors (Ambrose et al., 2016); however, the *P. pmonenusa* identified in the present study was resistant to colistin, ampicillin, vancomycin, meropenem, clindamycin, gentamicin, erythromycin, nitrofurantoin, rifampin, spectinomycin, and aztreonam, but not to cephem antibiotics or β-lactam/β-lactamase inhibitors.

Resistance to carbapenem antibiotics is known to result from mutations that affect the production of β-lactamase and the functional expression of efflux pumps, porins, and PBPs (Drlica and Zhao, 1997; Giske et al., 2008; Hashizume et al., 1984). There is an imminent need to study the underlying mechanism of antimicrobial resistance observed in meropenem-resistant *Acinetobacter junii*, *C. cucumeris*, *C. indologenes*, *Cupriavidus plantarum*, *E. anopheles*, *Elizabethkingi ameningoseptica*, *Elizabethkingi amiricola*, *Pandoraea pmonenusa*, *Pseudomonas pseuodelcali genes*, *Pseudomonas chengduensis*, *Pseudomonas tawnensis*, and *Stenotrophomonas maltophilia*, isolated in the present study.

Biofilms, sewage effluent sediments, wastewater treatment plant effluents, sewage sludge, pharmaceutical manufacturing plants, liquid manure tanks, and manure-fertilized soil are known to act as hot spots for the occurrence and spread of antibiotic resistance (Berkner et al., 2014). In South Korea, the industrial effluents are generally checked for total coliforms and ecotoxicity according to the classification of effluent discharge zones. Our results show that there is a need to perform toxicity tests and environmental hazard assessments on effluents containing antibiotics or antimicrobial substances, such as pharmaceutical industry discharge water, to prevent the occurrence and spread of antibiotic resistance (Hernando et al., 2006; Escher et al., 2011). In Korea, the ecotoxicity testing is performed by using water fleas (Hwang and Kim, 2018). It is believed that the toxicity testing of the effluents that include trace amounts of antimicrobial substances, too should be performed using microorganisms. The pharmaceutical industrial park that discharges wastewater into the river sampled in the present study has a wastewater treatment facility; however, there is a high probability of occurrence of antibiotic resistance due to an inflow of processed water containing various clinical disinfectants and discharge from antibiotic-manufacturing pharmaceutical factories. In general, the concentrations of antibiotics present in pharmaceutical effluents are significantly lower than the ones used in clinical practice (NIER, 2013). These concentrations, though not high enough to kill bacteria, are sufficient to exert selective pressure on bacteria to develop antimicrobial resistance (Gullberg et al., 2011). Therefore, it is necessary to implement proper treatment methods, including membrane filtration, ozonation, and UV disinfection, to completely remove the antibiotics and antibiotic-resistant bacteria present in pharmaceutical effluents to reduce their flow into the environment (Pruden et al., 2013).

**Conclusion**

The present study identified the species, contamination level, seasonal distribution, and antibiotic-resistance spectrum of meropenem-resistant bacteria in a river receiving pharmaceutical industry discharge. Our study showed that the presence of multi-drug resistant bacteria in the river water poses a threat to human health due to wider reach and use of river water. The outcomes of our study highlight the need to implement methods that can simultaneously disinfect multi-drug resistant bacteria and remove antibiotics from effluent containing discharge water from pharmaceutical and industrial units.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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