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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Effects of antibiotics in wastewater on the removal of human enteric viruses by specific interaction between virus and wastewater solids

研究課題名（英文）Effects of antibiotics in wastewater on the removal of human enteric viruses by specific interaction between virus and wastewater solids

研究代表者
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交付決定額（研究期間全体）：（直接経費）　　3,000,000円

研究成果の概要（和文）：アミカシンとクロラムフェニコールのEnterobacter cloacaeに対するMICをE－テストを使用して評価した。その後、細菌を抑制レベル以下の薬剤濃度で培養した。 SMP、LB-EPS、TB-EPSの生産を抽出して抽出された画分の炭水化物およびタンパク質濃度を測定した。励起発光マトリックス蛍光分光法によりEPS構造の変化を観察しました。その後、異なる薬剤濃度で増殖させたE.cloacaeの存在下でロタウイルスHAL1166の精密ろ過実験を行いました。
「水環境における薬剤耐性菌と薬剤耐性遺伝子によるヒトの健康リスクの理解：現在の知識と回答すべき質問」論文が公開された。

研究成果の学術的意義や社会的意義
副抑制薬剤濃度の存在下で、細菌細胞のEPS生産が増加しました。細菌、ウイルス、薬剤がリアクター内に共存しているため、EPS濃度の増加はウイルスの付着の増加を引き起こす可能性があります。ただし、EPSの量が増えると、膜反応器にファウリングが発生する可能性があります。したがって、ウイルスの付着を増やし、ファウリングを減らすための最適な条件を特定することが重要です。

研究成果の概要（英文）：We evaluated the MIC of amikacin and chloramphenicol to Enterobacter cloacae using E-test. After that we cultivated the bacteria under subinhibitory level antibiotics concentrations. We extracted the SMP, LB-EPS and TB-EPS production. Carbohydrate and protein concentrations of extracted fractions were measured. We observed changes in the EPS structure by Excitation Emission Matrix fluorescence spectroscopy. After that we conducted a microfiltration experiment of rotavirus HAL1166 in the presence of E.cloacae grown under different antibiotic concentrations.
A review paper titled "Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current knowledge and questions to be answered" is published in Critical Reviews in Environmental Science and Technology journal.

研究分野：土木環境システム関連

キーワード：Enteric virus　ARB　ARG　Specific interaction

１．研究開始当初の背景

Human enteric viruses including norovirus and rotavirus are major causes of non-bacterial epidemic gastroenteritis. Human excreta containing viruses end up in wastewater treatment plants. Major virus removal mechanisms involved in different wastewater treatment unit processes include adsorption to wastewater solids and subsequent sedimentation and inactivation, size separation, sunlight inactivation etc. Among all the virus removal mechanisms, adsorption to wastewater solids is commonly utilized in many types of treatment unit processes.

Virus can adsorb to wastewater solids specifically or non-specifically (Busscher and Weerkamp, 1987). Histo-blood group antigen (HBGA)-like substances present in bacterial extracellular polymeric substances (EPS) are shown to be a specific adsorbent for human norovirus and rotavirus (Miura, Sano, et al., 2013). However, there are other constituents in wastewater including antibiotics which can affect EPS concentration and structure. Studies have shown that increased antibiotic concentrations lead to increased EPS production by bacteria (Huang, Zhang, et al., 2014). Antibiotics can also interact with EPS via hydrophobic interactions (Khunjar and Love, 2011; Li, Pi, et al., 2016) and it can lead to loosened EPS structures which facilitates the mass transfer (Xu, Sheng, et al., 2013). Antibiotics removal efficiency by different wastewater treatment unit processes has revealed that the sorption to extracellular polymeric substances (EPS) and subsequent biodegradation as a major removal mechanism (Matos, Pereira, et al., 2014; Khunjar and Love, 2011).

２．研究の目的

Presence of antibiotics in wastewater, their interactions with EPS and the antibiotic induced structural changes in EPS raises questions regarding the consequences on the enteric virus removal due to specific interactions between viruses and the EPS. In this study we tried to elucidate the antibiotic induced effects on the human enteric virus removal by specific interactions.

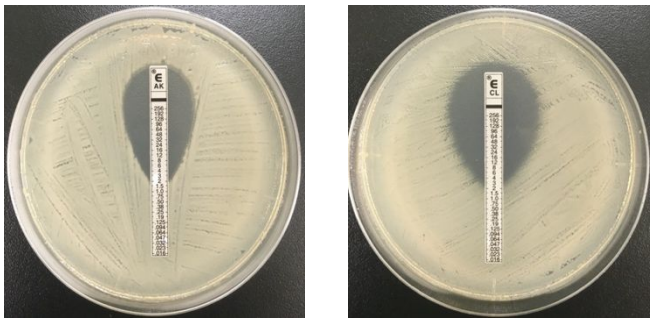
３．研究の方法

Enterobacter cloacae SENG-6 was used as the model bacterial strain in this study. Minimum inhibitory concentration (MIC) of *E. cloacae* SENG-6 was determined as 2 μg/ml for amikacin and chloramphenicol using E-test (Figure 1).

E. cloacae SENG-6 was cultivated at 37 ^{0}C overnight in Luria-Bertani medium in the presence of different antibiotic concentrations below the MIC. After cultivation, optical density (OD) at 600 nm for *E. cloacae* SENG-6 reduced from 1.84 (control) to

1.58 (1.9 μg/ml) for amikacin while the same OD value varied from 1.83 (control) to 1.26 (1.9 μg/ml) for chloramphenicol. Soluble microbial products (SMP), lightly bound extracellular polymeric substances (LB-EPS) and tightly bound EPS (TB-EPS) were extracted and quantity of carbohydrates and proteins were measured.

SMP and EPS were characterized excitation-emission matrix (EEM) spectra analysis, using a fluorescent spectrophotometer (F-7000, Hitachi, Japan).



Amikacin
MIC=2 μg/ml

Chloramphenicol
MIC=2 μg/ml

Figure 1: Determination of minimum inhibitory concentration (MIC) of amikacin and chloramphenicol for E. cloacae SENG-6

４．研究成果

Carbohydrate and protein concentrations were normalized using the OD value (Figure 2). Among the three fractions, highest protein concentration was present in SMP whereas the highest carbohydrate concentration was present in the TB-EPS fraction. Reduction of protein concentration in TB-EPS with increasing antibiotic concentration was evident in the presence of chloramphenicol while it was not apparent with amikacin.

Based on EEM results, cultivation of E. cloacae SENG-6 in the presence of antibiotics lead to changes in fluorescence components (Figure 3).

Change of EPS composition by the influence of antibiotics, may affect the formation of specific interactions and in turn affect the virus removal. Moreover, changes in the EPS composition can possibly change the properties of gel/cake layers produced by the bacteria. Therefore, antibiotics in wastewater may affect the virus removal efficiencies of reactors based on biofilms. The real consequences of EPS quantity and composition changes on the removal of human enteric viruses by specific interactions with HBGA-positive bacteria needed to be further elucidated.

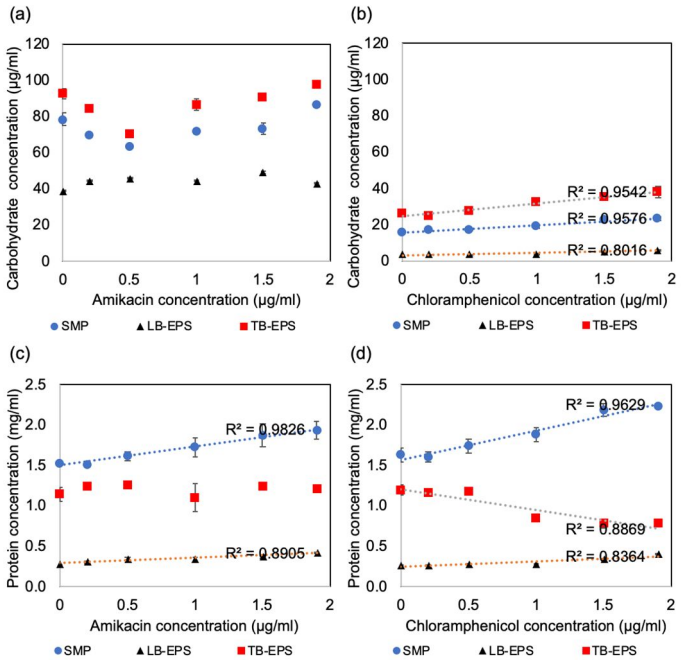


Figure 2: Change of carbohydrate (a and b) and protein (c and d) concentration present in the extracted SMP, LB-EPS and TB-EPS of E. cloacae SENG-6 cultivated with different amikacin and chloramphenicol concentrations

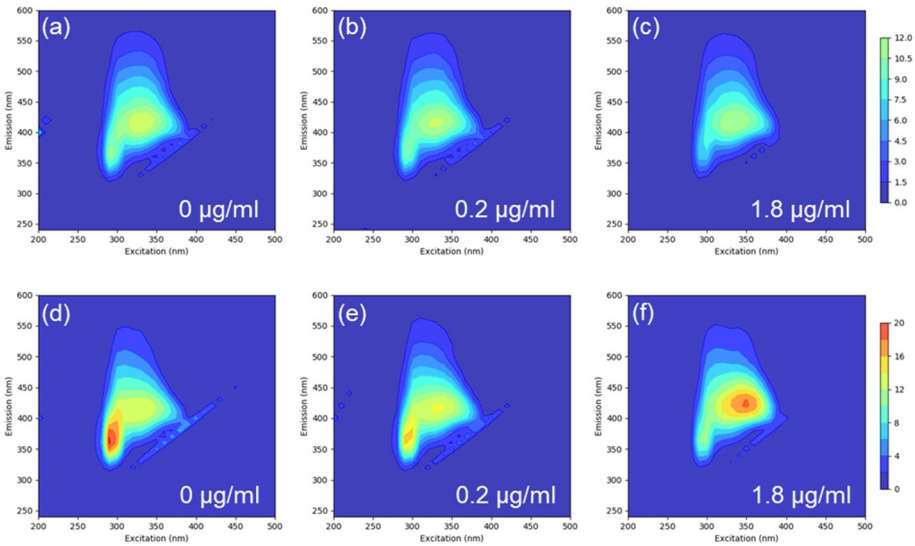


Figure 3: EEM fluorescence spectra of SMP extracted from E. cloacae SENG-6 cultivated with different amikacin (a-c) and chloramphenicol (d-f) concentrations

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Xu, J., Sheng, G. P., Ma, Y., Wang, L. F., and Yu, H. Q. (2013) Roles of extracellular polymeric substances (EPS) in the migration and removal of sulfamethazine in activated sludge system. Water Research, 47(14), 5298–5306.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Amarasiri Mohan、Sano Daisuke、Suzuki Satoru	４．巻 50
２．論文標題 Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Current knowledge and questions to be answered	５．発行年 2019年
３．雑誌名 Critical Reviews in Environmental Science and Technology	６．最初と最後の頁 1～44
掲載論文のDOI（デジタルオブジェクト識別子） 10.1080/10643389.2019.1692611	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計6件（うち招待講演　2件／うち国際学会　3件）

１．発表者名 小沼千紘、モハン　アマラシリ、門屋俊祐、佐野大輔
２．発表標題 細胞外薬剤耐性遺伝子の遊離塩素による消毒効果の評価
３．学会等名 平成30年度土木学会東北支部・技術研究発表会
４．発表年 2019年

１．発表者名 小沼千紘、モハン　アマラシリ、鈴木聡、佐野大輔
２．発表標題 水環境サンプルからの形質転換可能な細胞外薬剤耐性遺伝子の回収方法の確立
３．学会等名 第54回日本水環境学会年会
４．発表年 2020年

１．発表者名 Mohan Amarasiri, Chihiro Konuma and Daisuke Sano
２．発表標題 Extracellular antibiotic resistance gene decay by free chlorine treatment
３．学会等名 20th IWA Symposium on Health Related Water Microbiology（国際学会）
４．発表年 2019年

１．発表者名
Mohan Amarasiri

２．発表標題
Quantification of human health risks caused by antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: Future research directions

３．学会等名
The 6th Environmental Technology and Management Conference（招待講演）

４．発表年
2019年

１．発表者名
Mohan Amarasiri, Hui Cheng, Yu-You Li, Daisuke Sano

２．発表標題
Antibiotic-induced Changes in Enterobacter cloacae SENG-6 Extracellular Polymeric Substances

３．学会等名
8th IWA Microbial Ecology and Water Engineering Specialist Conference（国際学会）

４．発表年
2019年

１．発表者名
Mohan Amarasiri

２．発表標題
Watewater Treatment Technologies for ARG Removal

３．学会等名
WHO-HRWM workshop on antimicrobial resistance（招待講演）（国際学会）

４．発表年
2019年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考