HOKKAIDO UNIVERSITY

Title	Ciliates promote the transfer of a plasmid encoding blaNDM-5 from Escherichia coli, isolated from a hospital in Japan, to other human pathogens
Author(s)	Okubo, Torahiko; Matushita, Mizue; Ohara, Yukiko et al.
Citation	International Journal of Antimicrobial Agents, 49(3), 387-388 https://doi.org/10.1016/j.ijantimicag.2017.01.003
Issue Date	2017-03
Doc URL	https://hdl.handle.net/2115/68719
Rights	©2017, Elsevier. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/
Rights(URL)	https://creativecommons.org/licenses/by-nc-nd/4.0/
Type	journal article
File Information	Int. J. Antimicrob. Agents.2017.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Ciliates promote the transfer of plasmid encoding *bla*$_{\text{NDM-5}}$ from *Escherichia coli*, isolated from a hospital in Japan, to other human pathogens

Torahiko Okubo[a]

Mizue Matushita[a]

Yukiko Ohara[a]

Junji Matsuo[a]

Satoshi Oguri[b]

Tatsuya Fukumoto[b]

Kasumi Hayasaka[b]

Kouzi Akizawa[b]

Hitoshi Shibuya[b]

Chikara Shimizu[b]

Hiroyuki Yamaguchi[a,*]

[a]*Department of Medical Laboratory Science, Faculty of Health Sciences, Hokkaido University Graduate School of Health Sciences, Sapporo, Japan*

[b]*Hokkaido University Hospital, Sapporo, 060-8648, Japan*

*Corresponding author. Present address: Department of Medical Laboratory Science, Hokkaido University, Nishi-5 Kita-12 Jo Kitaku, Sapporo 060-0812, Japan.

Tel/Fax: +81-11-706-3326

E-mail address: hiroyuki@med.hokudai.ac.jp

Sir,

The rapid global dissemination of New Delhi metallo-β-lactamase (NDM)-producing isolates is unquestionably a major concern to public health. However, this is a significant challenge for controlling the emergence of multidrug-resistant bacteria leading to hospital or community-acquired infections [1]. Ciliates (*Tetrahymena*), which are ubiquitous in natural environments such as moist soil or pond water [2], facilitate the transfer of genes encoding extended-spectrum β-lactamases (ESBLs) between *Escherichia coli* strains [3], by conjugation via vesicle accumulation [4]. We therefore assessed if ciliates could promote gene transfer from NDM ($bla_{\text{NDM-5}}$)-producing *E. coli* 9/III strain isolated at a hospital in Japan to other *E. coli* or *Salmonella*.

E. coli strain 9/III was isolated in 2016 from a fecal sample from a 20-year-old woman with hematologic disease hospitalized at Hokkaido University Hospital. The patient had traveled abroad but had not visited India or the Middle East. This strain exhibited carbapenem resistance, as confirmed by a screening test in the hospital's bacteriological examination laboratory. According to the CLSI guideline [5], the isolate

was determined to be resistant with cefotaxime (CTX) (>256 mg/L), ceftazidime (CAZ) (>256 mg/L), imipenem (IPM) (32 mg/L), tetracycline (TET) (256 mg/L), and ciprofloxacin (CIP) (>256 mg/L), but not to tigecycline (TIG) (1 mg/L) and colistin (COL) (2 mg/L). The phylogenic analysis of the β-lactamase genes revealed that *E. coli* 9/III carried two distinct β-lactamase genes, $bla_{\text{NDM-5}}$ (Accession number: LC189236) and $bla_{\text{CTX-M-15}}$ (Accession number: LC189237). The *tet*(A) gene that mediates resistance to TET was detected. Also, mutations in GyrA (S83L, D87N), ParC (S80I), and ParE (S458A) were detected that are characteristic of CIP resistance. O serotyping using the Denka-Seiken kit No.1, large-scale population structure analysis, and multilocus sequence typing (MLST) analysis revealed that *E. coli* strain 9/III was characterized as OUT, A_1, and ST410, respectively. Also, S1-PFGE analysis revealed that *E. coli* 9/III carried two plasmids, of approximately 45 kb and 130 kb (data not shown). The transferability of carbapenem-resistance determinants was determined by conjugation experiments as previously established [3, 4]. In brief, equal numbers (10^9 CFU/mL) of *E. coli* 9/III as a donor and *E. coli* ML4909 or *Salmonella* ser. Enteritidis as a recipient were mixed in fresh Page's amoeba saline with or without ciliates

58 [*Tetrahymena thermophila* (Tth) and *Tetrahymena* sp. (Tsp)] (10^5 cell/mL) and
59 incubated overnight at 30ºC. The transconjugants were detected as blue colonies on
60 modified Drigalski lactose agar (for *E. coli*) or black colonies on MLCB agar (for
61 *Salmonella*) containing CAZ (10 mg/L), and the presence of carbapenem-resistance
62 determinants was also confirmed by PCR. The gene transfer frequency was expressed as
63 the number (CFU) of transconjugants per recipient. Both recipients, *E. coli* strain
64 ML4909 and *Salmonella* Enteritidis, showed susceptibility to all of the antibiotics used
65 for the study. Both *E. coli* strain 9/III and *Salmonella* Enteritidis are clinical strains
66 originally isolated in Hokkaido University Hospital. Also, *E. coli* ML4909 was kindly
67 provided from Dr. Tamura, Rakuno Gakuen University, Japan.

68 The frequency of transconjugants in the absence of ciliates was 8.2×10^{-6} (*E. coli*
69 ML4909) (Fig. 1A, left panel) and 2.4×10^{-8} (*Salmonella* Enteritidis) (Fig. 1A, right
70 panel). By contrast, the frequency of transconjugants in the presence of ciliates was 2.6
71 $\times 10^{-4}$ (Tsp) ($p<0.05$) or 1.3×10^{-5} (Tth) ($p=0.563$) (Fig. 1A, left panel) for *E. coli*
72 ML4909, and 5.8×10^{-6} (Tsp) ($p<0.05$) or 5.3×10^{-6} (Tth) ($p<0.05$) (Fig. 1A, right
73 panel) for *Salmonella* Enteritidis. Giemsa staining revealed that in the mixed culture

with the bacteria ciliates formed a large number of vacuoles into the cells ciliates with the vacuoles packed by the bacteria, indicating the presence of bacteria into ciliates (Fig. 1B, See arrows). In addition, in the presence of ciliates, the bacteria could survive, however, with a decrease of bacterial number, approximately 3-log CFUs. Also, the number of ciliates into the mixed culture with the bacteria was more or less constant during culture period. Successful conjugations by both of the bacteria were confirmed by PCR using primers specific to the $bla_{\text{NDM-5}}$ gene sequence, but not the $bla_{\text{CTX-M-15}}$ gene sequence (data not shown). The antimicrobial susceptibilities of transconjugants derived from *E. coli* ML4909 were similar to those of the donor *E. coli* 9/III strain: CTX (8 mg/L), CAZ (128 mg/L), IPM (4 mg/L), TET (1 mg/L), CIP (0.25 mg/L), TYG (0.5mg/L), and COL (0.25 mg/L). Also, the transconjugant strain derived from *Salmonella* Enteritidis showed resistance to β-lactams [CTX (>128 mg/L) and CAZ (>128 mg/L)], but not to TET (1 mg/L) and CIP (≤ 0.03125 mg/L). Since the transferable plasmid was found to be small (approximately 45 kb) (data not shown), this suggested that it carried the $bla_{\text{NDM-5}}$ gene, which was transferred to the transconjugants. Also, since neither resistance to TET or CIP was detected in the transconjugants, these

resistant genes [*tet*(A) and *gyrA*] were likely encoded on the genome. The frequency of gene transfer with *Salmonella* significantly increased in the presence of ciliates, compared with that of the *E. coli* recipient. *Salmonella*, which is a facultative intracellular pathogen, might live longer than *E. coli* in the vacuoles of ciliates. Also, no increase in the gene transfer frequency with an *E. coli* recipient was seen in the presence of Tth ciliates. The combination between bacteria and ciliates may be an important factor in determining the frequency of gene transfer among bacteria in the presence of ciliates.

Thus, environmental niches in which ciliates accumulate may be a hotspot facilitating the emergence of NDM-producing bacteria due to the promotion of gene transfer among human pathogenic bacteria.

Funding

This work was supported by Management Expenses Grants for National University, Japan

Competing interests

None declared.

Ethical approval

Not required.

111 ## References

112 [1] Aminov RI. Horizontal gene exchange in environmental microbiota. *Front*
113 *Microbiol* 2011;**2**:158.

114 [2] Acosta-Mercado D, Lynn DH. The edaphic quantitative protargol stain: a sampling
115 protocol for assessing soil ciliate abundance and diversity. *J Microbiol Methods*
116 2003;**53**:365-75.

117 [3] Oguri S, Matsuo J, Hayashi Y, Nakamura S, Hanawa T, Fukumoto T, et al. Ciliates
118 promote the transfer of the gene encoding the extended-spectrum β-lactamase
119 CTX-M-27 between *Escherichia coli* strains. J Antimicrob Chemother
120 2011;**66**:527-30.

121 [4] Matsuo J, Oguri S, Nakamura S, Hanawa T, Fukumoto T, Hayashi Y, et al. Ciliates
122 rapidly enhance the frequency of conjugation between *Escherichia coli* strains
123 through bacterial accumulation in vesicles. *Res Microbiol* 2010;**161**:711-9.

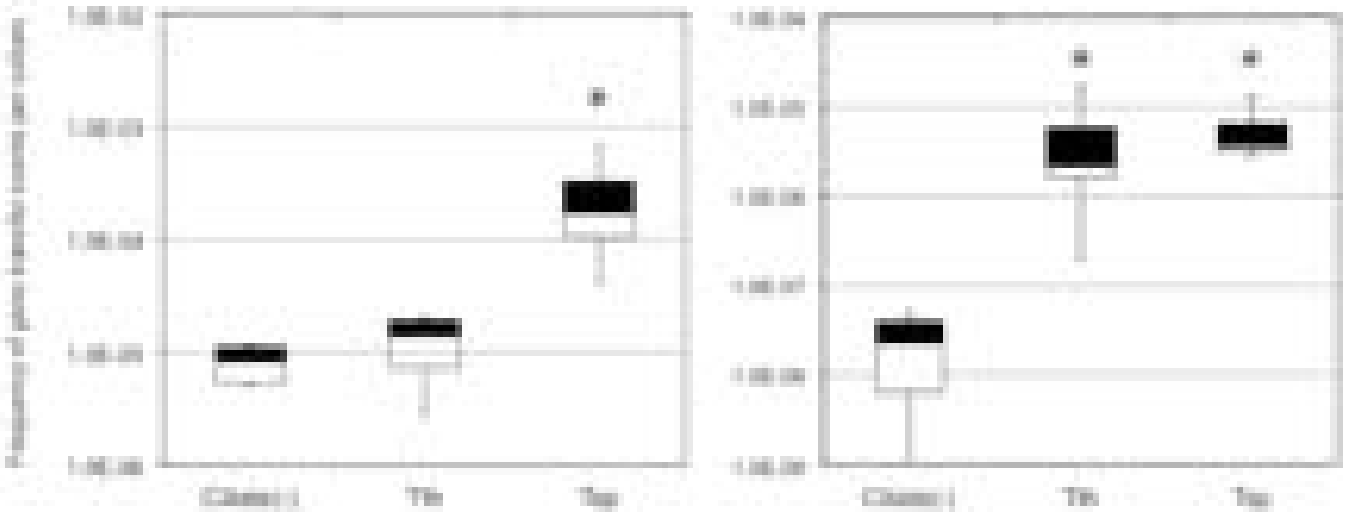
124 [5] CLSI. 2012: Performance standards for antimicrobial susceptibility testing; 22nd
125 informational supplement. CLSI document M100-S22. Clinical and Laboratory
126 Standards Institute, Wayne, PA.

Figure Legend

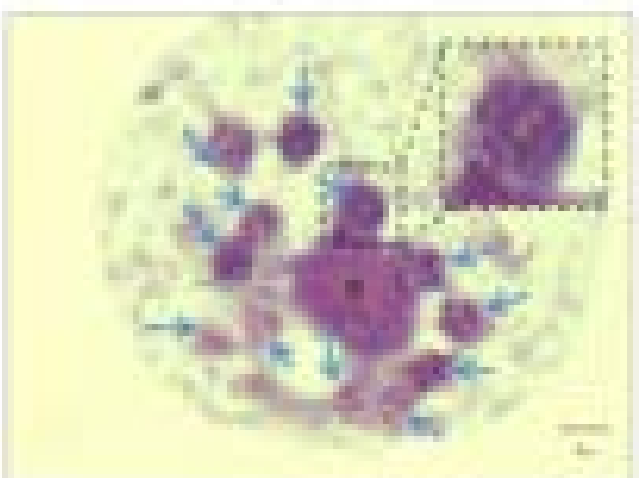
Fig. 1. Effect of ciliates on the conjugation frequency of NDM-producing *E. coli* strain 9/III. (A) Conjugation frequency of *E. coli* 9/III to *E. coli* ML4909 (left panel) or *Salmonella* Enteritidis (right panel) in the presence or absence of ciliates (Tsp: *Tetrahymena* sp.; Tth: *Tetrahymena thermophila*). Conjugation frequency was estimated as the number of transconjugants for each recipient. Data show averages of the conjugation frequency ± standard deviation. Statistical analysis was performed using the Mann-Whitney test and a p value of <0.05 was considered significant. *, $p< 0.05$ vs. values for bacteria alone. (B) Representative Giemsa-stained images showing the vacuoles of ciliates packed with the bacteria. Arrows show the vacuoles. Square surrounded by dashed line are enlarged to define the bacterial accumulation in vacuole. N, nucleus. Magnification, × 1,000.

FIG 1

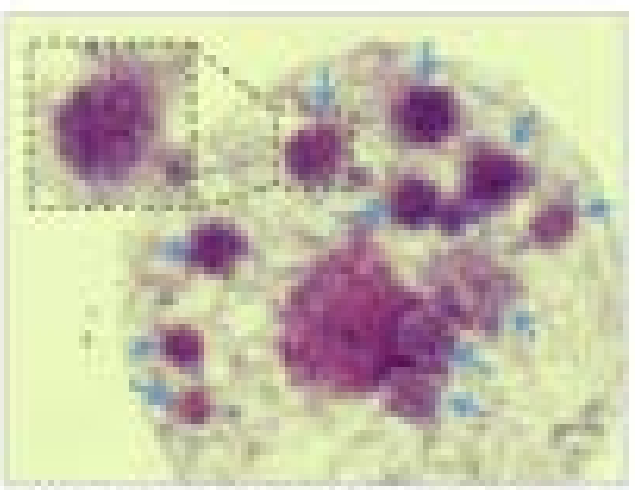
A



B



With *E. coli* strain Nissle and *E. coli* ML4909



With *E. coli* strain Nissle and *Salmonella Enteritidis*