

Short Communication

Characterization of the First Korean Isolate of a *Chlamydia pneumoniae* Strain

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SUMMARY: *Chlamydia pneumoniae* is a common pathogen that causes upper and lower respiratory tract infections and is difficult to isolate from clinical specimens. Recently, we succeeded in isolating the first *C. pneumoniae* strain in Korea. This study characterizes the morphology, infectivity, and drug sensitivity of the Korean strain, designated LKK-1. Electron microscopy was performed for thin sections, and the infectivity over time was tested by counting the inclusion-forming units every 12 h. The minimum inhibitory concentrations of doxycycline, erythromycin, clarithromycin, ciprofloxacin, and levofloxacin were determined following the standard Japanese method. The elementary bodies of LKK-1 were round, like those in Japanese strain KKpn-1, whereas those of TW-183 have wavy cell membranes and are pear-shaped. The infectivity curve and drug sensitivities of LKK-1 were nearly the same as those of KKpn-1. In conclusion, LKK-1, the first strain from Korea, is similar to the Japanese strain KKpn-1 in terms of morphology, growth, and drug sensitivities, and shows a distinct difference in morphology compared with TW-183. Further studies are needed to elucidate the morphological differences between round strains and classical pear-shaped strains of *C. pneumoniae*.

Chlamydia pneumoniae is a well-known pathogen with a spectrum of clinical manifestations including upper and lower respiratory tract infections, and it has recently been tentatively linked to atherosclerosis (1-5). *C. pneumoniae* is notoriously difficult to culture using cell-culture systems, and few laboratories have successfully isolated it from patients (6,7). Recently, we succeeded in isolating a *C. pneumoniae* strain from a 22-year-old woman with acute pharyngitis. This strain, designated LKK-1, is the first isolate from Korea (8). Morphologically, *C. pneumoniae* has a wavy outer membrane and is described as "pear-shaped". In contrast, isolates from Japan are reported to have round outer membranes (9-11).

This study characterizes the newly isolated Korean strain and compares its morphology, infectivity, and susceptibility to antimicrobial agents with those of known strains.

The strains of *C. pneumoniae* used in this study were TW-183 (ATCC # VR-2282), KKpn-1 (provided by the National Institute of Infectious Diseases, Tokyo), and LKK-1 (the first Korean strain).

To prepare specimens for electron microscopy, monolayer cultures of HEp-2 cells on coverslips (13.5 mm in diameter) were inoculated, incubated, and collected as reported previously (11), and thin sections were prepared (12). The outer membranes were negatively stained with 1% phosphotungstic acid solution.

To evaluate the infectivity of LKK-1 over time, HEp-2 cells were used and a previously described method was followed (13). Infected cells were harvested at 12, 24, 36, 48, 72, 84, 96, 108, and 120 h post-inoculation, and re-inoculated on HeLa 229 cells; inclusion-forming units were counted using a previously reported method (14).

Drug-susceptibility testing of LKK-1 and KKpn-1 followed

the standard methods described by the Japanese Society of Chemotherapy (14). The antimicrobials tested were doxycycline (Joongoe Pharmaceutical Co., Seoul, Korea), erythromycin (Abbott Korea Co., Seoul, Korea), clarithromycin (Abbott Korea), ciprofloxacin (Ildong Pharmaceutical Co., Seoul, Korea), and levofloxacin (Jeil Pharmaceutical Co., Seoul, Korea).

In thin sections seen under an electron microscope, the EBs of LKK-1 and KKpn-1 appeared round and had a narrow periplasmic space (Fig. 1), whereas the EBs of strain TW-183 had a wide periplasmic space with a wavy outer membrane, forming a pear-shape. The morphologies of the reticulate bodies were indistinguishable among the three strains.

The infectivity of LKK-1 was nearly the same as that previously reported by Miyashita et al. (13). The infectivity of LKK-1 increased sharply from 36 h after inoculation and reached a plateau at approximately 60 h, which was maintained for another 60 h (Fig. 2).

Table 1 shows the susceptibility profiles of LKK-1 and KKpn-1. The minimum inhibitory concentrations (MICs) of these two strains were nearly the same.

This study characterized the newly isolated Korean strain of *C. pneumoniae*, comparing it with several other strains. The morphology of the outer membrane of the elementary bodies (EBs) of LKK-1 was round, and appeared to be nearly the same as that of the Japanese strain, KKpn-1, whereas the EBs of ATCC strain TW-183 appeared pear-shaped. When *C. pneumoniae* was first identified as a new species, the pear-shaped outer membranes of the EBs were thought to be characteristic of this species (15,16). However, strains with EBs with round outer membranes have been isolated from an Iranian and a Finn (17,18). Further, one Japanese strain, designated AC-43, was reported to be pear-shaped (19). The reason for this morphological difference among strains is not clear. The protein profiles of EBs of both the Japanese and TW-183 strains are reported to be identical (13); therefore, the morphological difference does not appear to originate from

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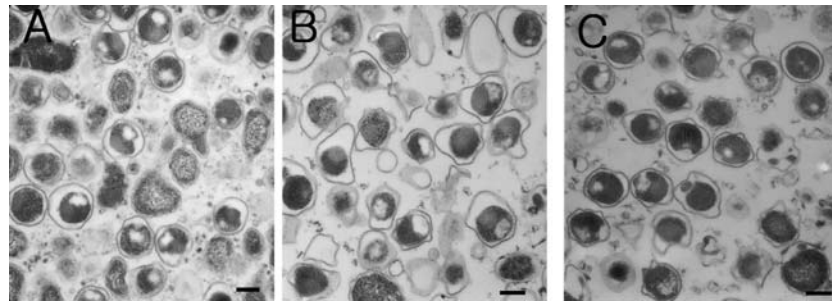


Fig. 1. Thin sections of *C. pneumoniae* LKK-1, TW-183, and KKpn-1 in HEp-2 cells 72 h post-inoculation. A: Strain LKK-1; B: TW-183; C: KKpn-1. EBs of both LKK-1 and KKpn-1 have a narrow periplasmic space and are round, whereas those of TW-183 have a wider periplasmic space and wavy outer membranes (i.e., they are pear-shaped). ($\times 30,000$, Bars indicate 250 nm)

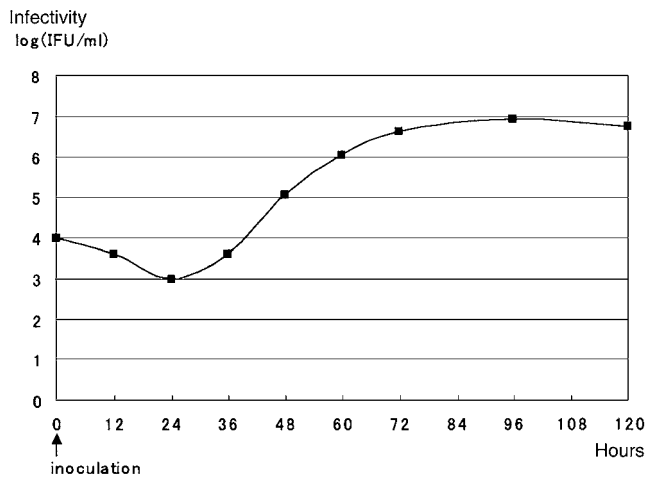


Fig. 2. Growth curve of strain LKK-1 in HEp-2 cells. IFU; Inclusion Forming Unit.

Table 1. MICs ($\mu\text{g}/\text{mL}$) of antimicrobial agents against strains LKK-1 and KKpn-1

	LKK-1	KKpn-1
Doxycycline	0.063	0.063
Erythromycin	0.5	0.5
Clarithromycin	0.031	0.016
Ciprofloxacin	0.5	0.5
Levofloxacin	0.25	0.25

a difference in protein composition. Further studies are needed to elucidate the protein profile of strain LKK-1.

Although LKK-1 might be found to be a contaminant from laboratory work using Japanese strains, at the time of isolation there were no Japanese *C. pneumoniae* strains in our laboratory, and previous laboratory work had involved only TW-183. Therefore, the LKK-1 strain was not considered a contaminant.

With respect to infectivity and drug susceptibility, there were no differences between LKK-1 and KKpn-1.

In conclusion, the first Korean isolate of a *C. pneumoniae* strain showed a round morphology like that of a known Japanese strain, and had drug sensitivities and a growth curve similar to those of the Japanese strain.

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