

wounds (6%). Community- and hospital-isolated strains represented 55% and 45% of total isolates, respectively. Colonized isolates (78%) were more frequent than infected isolates (21%). All isolates were categorized as having penicillin G MIC \leq 0.03 $\mu\text{g}/\text{mL}$ using an automated system; MICs determined based on the microdilution method were 0.016 $\mu\text{g}/\text{mL}$ (2%), 0.03 $\mu\text{g}/\text{mL}$ (44%), and 0.06 $\mu\text{g}/\text{mL}$ (54%) (Table). Notably, no isolates harbored the *blaZ* gene. The results from the nitrocefin-based and zone-edge tests were consistent with those obtained by PCR.

Conclusion. *S. aureus* isolates with penicillin G MIC \leq 0.03 $\mu\text{g}/\text{mL}$ exhibited a low frequency of β -lactamase production. Thus, screening for β -lactamase production may be unnecessary for isolates showing such high-susceptibility.

Table: Prevalence of β -lactamase production in 108 *S. aureus* isolates with associated penicillin G MIC

Penicillin G MICs ($\mu\text{g}/\text{mL}$)	n (%)	<i>blaZ</i> -positive by PCR, n (%)
0.004	0 (0)	0 (0)
0.008	0 (0)	0 (0)
0.016	2 (2)	0 (0)
0.03	48 (44)	0 (0)
0.06	58 (54)	0 (0)
0.12	0 (0)	0 (0)

MIC, minimum inhibitory concentration

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2043. Rapid and Specific Detection of *Escherichia coli* Sequence Type 131 (ST131) and its Key Subclones Using a Novel Single-tube Multiplex PCR Assay

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Session: 234. Diagnostics - Bacterial Identification and Resistance

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Background. *E. coli* ST131 is a recently emerged, pandemic multidrug-resistant pathogen. ST131 comprises multiple distinctive sub-clones that differ by serotype, *fimH* (type-1 fimbriae adhesin) allele, resistance phenotype and genotype, and host group predilection. PCR assays have been described for detecting ST131 and several of its subclones, but not all in single reaction.

Methods. To create a single-tube multiplex PCR assay for detecting key ST131 subclones and their associated O types, 2 novel primers were combined with 9 published primers. The primers target allele-specific SNPs in *mdh36* and *gyrB4* (from multi-locus sequence typing), *fimH30*, *sbmA* (transport protein) and *rfb* O16 and O25b specific nucleotide sequence. The resulting band combinations allow resolution of ST131 per se and of several well established ST131 sub-clones, including ST131 O16 (a.k.a. H41 or clade A) and 4 variants of ST131 O25b, i.e., non-H30 (a.k.a. H22 or clade B), H30 (a.k.a. clade C), H30 non-Rx (a.k.a. H30R1 or clade C₂), and H30Rx (a.k.a. clade C₃), which can occur with either *fimH30* or an alternate *fimH* allele (typically *fimH35*). Primers were designed to have a common annealing temperature and were tested on different thermal cyclers

Results. The ST131 multiplex assay identified correctly by subclone (according to the whole genome phylogeny of Price et al.) all 104 ST131 strains (100%) from Price et al. Additionally, in blinded testing of 90 fresh consecutive *E. coli* clinical isolates, for assigning isolates to ST131 and to its key subclones the new assay yielded 100% concordance with the published single PCR assays that were run in parallel. Assay performance was consistent across thermal cyclers. Figure 1 demonstrates the different unique PCR profiles.

Figure 1: PCR product of 5 ST131 sub-clones and 2 non-ST131.



Conclusion. This novel ST131 multiplex PCR assay provides a rapid and specific single-step diagnostic tool for efficiently assigning *E. coli* isolates to ST131 and its key subclones. It should prove useful for epidemiological studies and potentially clinical diagnostics.

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2044. Epidemiological and Molecular Characteristics of Carbapenemase-Producing *Enterobacteriaceae* in a Tertiary Hospital in Korea: Possible Emergence of KPC-Producing *Escherichia coli* ST471 strain

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Background. Carbapenem-resistant *Enterobacteriaceae* (CRE) and especially carbapenemase-producing *Enterobacteriaceae* (CPE) were rare in Korea but have been rapidly increasing since 2015. CPE outbreaks are now common among Korean hospitals. We aimed to investigate the epidemiology and molecular characteristics of CPE clinical isolates at a tertiary hospital in the southeastern part of Korea.

Methods. CRE isolates, which had been isolated and stored from diagnostic or surveillance culture specimens between January 2011 and February 2017, were investigated. Multiple isolates from a patient were included in the study when they are of different species or separated by 30 days or more. Antibiogram was generated by the Vitek 2 system. Multiplex PCR was performed to detect 5 types of carbapenemases (KPC, NDM, VIM IMP, OXA). Of the PCR-positive CPE isolates, multi-locus sequence typing was performed for *E. coli*, *K. pneumoniae* and *E. cloacae*. For the patients with CPE, information on admission period, ward, room, bed position and common medical staff was collected to find epidemiologic link.

Results. Total 197 CRE isolates from 169 patients were investigated, and 65 isolates were CPE. The earliest CPE isolate was in January 2016. Of the CPE isolates, the most common species were *E. coli* (45%) and *K. pneumoniae* (43%), the most common carbapenemase types were KPC (80%) and NDM (14%). Two CPE strains with KPC, *E. coli* ST471 (18%) and *K. pneumoniae* ST307 (25%), were prevalent. KPC was mostly associated with *K. pneumoniae* (27/52) and *E. coli* (19/52), and NDM with *E. coli* (8/9) and *K. pneumoniae* (1/9). Between August 2016 and February 2017, there were 32 outbreak-associated CPE isolates from 9 clusters (5 *E. coli* clusters, 3 *K. pneumoniae* ones, 1 *E. cloacae* one). The largest cluster was of 7 patients from whom KPC-2-positive *E. coli* ST471 lineage was isolated.

Conclusion. Species of the CPE isolates in our hospital were mostly *E. coli* and *K. pneumoniae*, and KPC was the most common type of carbapenemase. KPC-positive *E. coli* ST471, not well-known in Korea and East-Asia, may be a newly emerging CPE strain. Although *E. coli* is known to only rarely cause CPE outbreaks, it might commonly cause in CPE outbreaks in the southeastern part of Korea.

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2045. Molecular Serotype-specific Identification of *Haemophilus influenzae* Using the Loop-mediated Isothermal Amplification Method

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Background. Over the past several decades, the incidence of meningitis caused by *Haemophilus influenzae* in children has decreased due to wide-spread vaccination of *H. influenzae* type b (Hib). The incidence of invasive diseases due to *H. influenzae* that were not included in the vaccines, however, has increased. We developed a rapid, simple, and cost-effective method for detecting serotypes of *H. influenzae*.

Methods. We designed LAMP primer sets based on the sequences available for the capsular types a, c, d, e, and f. The assays were evaluated regarding reactivity, specificity and sensitivity of the assays. To be used for the patients suspected meningitis, we evaluated the detection limit of the non-Hib serotype specific LAMP assay using DNAs spiking CSF specimens.

Results. The reactivity and specificity of the LAMP assays were confirmed using 6 serotypes and nontype of *H. influenzae* strains, plus 21 strains representing other *Haemophilus* species and non-*Haemophilus* genera. The sensitivity of the LAMP assays was confirmed from 10 to 10³ copies per reaction that was 1 to 10³ times of sensitivity of the non-Hib serotype specific PCR that demonstrated the sensitivity 10³ or 10⁴ genome copies per reaction. The sensitivity of the LAMP was also the same, 10 to 10³ genome copies per reaction.

Conclusion. This is the first report of the serotype-specific identification assay for *H. influenzae* using the LAMP method. Our results suggest potential usage for children or adults with suspected meningitis in resource limited laboratories or public health surveillance systems.

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