**Background.** CRE infections cause significant mortality, in large part because rapid identification of these infections is challenging. We tested the hypothesis that CRE and their isogenic carbapenem-susceptible counterparts have differential metabolic responses to carbapenem therapy.

**Methods.** We generated isogenic pairs of *E. coli*, *E. cloacae*, and *K. pneumoniae* by inserting a *bla*<sub>NDM-1</sub>-containing plasmid into carbapenem-susceptible *E. coli*, *E. cloacae*, and *K. pneumoniae*. We confirmed phenotypic meropenem (MPM) resistance per CLSI breakpoints for Enterobacteriaceae (MIC ≥2) in the NDM-1 member and susceptibility (MIC<1) in the NDM-1 member of each pair. We administered 2×10<sup>6</sup> CFU of each isolate intranasally to 23–28 g male C57BL/6J mice, infecting 6 mice with the NDM-1 member and 6 with the NDM-1 member of each species pair (12 mice per bacterial species). 24 hours after infection, we treated 3 mice in each NDM-1- and NDM-1- bacterial species cohort with MPM over 4 hours, and the other 3 mice in each cohort with saline over 4 hours as controls, confirming adequate infection (a target of 10<sup>6</sup> CFU of lung tissue) in quantitative lung homogenate cultures. We then collected breath samples from each mouse via tracheostomy using a murine ventilator, identifying all volatile metabolites in each sample using thermal desorption-gas chromatography/tandem mass spectrometry. We used Wilcoxon tests to examine differences in metabolite abundance between MPM and saline-treated control mice in the NDM-1 and NDM-1- member of each species pair, with a two-sided P-value threshold of <0.1.

**Results.** Several breath volatile metabolites changed differentially within each NDM-1'/NDM-1 pair, outlined in Table 1 (*E. coli*), Table 2 (*E. cloacae*), and Table 3 (*K. pneumoniae*). Each listed metabolite that changed with MPM did not change with MPM in mice infected with each isogenic counterpart.

**Conclusion.** There are differential in vivo metabolic responses with effective vs. ineffective treatment of mice with pneumonia caused by *E. coli*, *E. cloacae*, and *K. pneumoniae* pairs that are genetically identical other than the *bla*<sub>NDM-1</sub>; this differential treatment response can potentially be used to identify these infections.

| Table 1. Differentially changing breath metabolites in mice infected with NDM-1*<sup>+</sup> vs. NDM-1- *Escherichia coli* |
|-----------------|-----------------|-----------------|
| **Breath Volatile Metabolite** | **Escherichia coli** | **Escherichia coli** |
| **Direction of change with MPM treatment** | **Microbe** | **Direction of change with MPM treatment** | **Microbe** |
| 2-methyl-6-oxo-hexanoic acid | Increased | 2-methyl-6-oxo-hexanoic acid | Increased |
| 2,4-dimethyl-4,5-hexadiene | Increased | 2,4-dimethyl-4,5-hexadiene | Increased |
| Camphor | Increased | Camphor | Increased |
| 2,6,10-trimethyl-dodecanol | Increased | 2,6,10-trimethyl-dodecanol | Increased |
| Undecanol | Increased | Undecanol | Increased |
| Octanal | Increased | Octanal | Increased |
| 3-methyl-2-butenal | Increased | 3-methyl-2-butenal | Increased |
| Acetaldelyde | Increased | Acetaldelyde | Increased |

*MPM: meropenem, ND: no difference in abundance between MPM and saline treatment*

**Table 2. Differentially changing breath metabolites in mice infected with NDM-1*<sup>+</sup> vs. NDM-1- *Enterobacter cloacae***

| **Breath Volatile Metabolite** | **Enterobacter cloacae** | **Enterobacter cloacae** |
|-----------------|-----------------|-----------------|
| **Direction of change with MPM treatment** | **Microbe** | **Direction of change with MPM treatment** | **Microbe** |
| 2,3,7-trimethyl-decanol | Increased | 2,3,7-trimethyl-decanol | Increased |
| 2,4-dimethyl-heptadecene | Increased | 2,4-dimethyl-heptadecene | Increased |
| 3-methyl-dodecanol | Increased | 3-methyl-dodecanol | Increased |
| 4-ethyl-lactic acid | Increased | 4-ethyl-lactic acid | Increased |
| 2,6,10-trimethylnonadecan-1-ol | Increased | 2,6,10-trimethylnonadecan-1-ol | Increased |
| 2,4-dimethyl-6-butyrolactone | Increased | 2,4-dimethyl-6-butyrolactone | Increased |
| 3-methyl-dodecanol | Increased | 3-methyl-dodecanol | Increased |
| Benzyl butyrate | Decreased | Benzyl butyrate | Decreased |
| Undecenal | Decreased | Undecenal | Decreased |
| 1-methyl-nonylbenzene | Increased | 1-methyl-nonylbenzene | Increased |
| butylcyclohexane | Increased | butylcyclohexane | Increased |
| 2-ethyl-4-tert-butylcyclohexane | Increased | 2-ethyl-4-tert-butylcyclohexane | Increased |
| Decane | Increased | Decane | Increased |
| 1,3-Octadiene | Increased | 1,3-Octadiene | Increased |
| Undecenal | Increased | Undecenal | Increased |
| 2-methylpropanol methyl hydrogen | Increased | 2-methylpropanol methyl hydrogen | Increased |
| 2-ethyl-4-tert-butylcyclohexane | Increased | 2-ethyl-4-tert-butylcyclohexane | Increased |
| Sulfuric acid, cyclohexylmethyl tridecyl ether | Increased | Sulfuric acid, cyclohexylmethyl tridecyl ether | Increased |
| 1-methyl-3-buten-1-ol | Increased | 1-methyl-3-buten-1-ol | Increased |
| 2,6,10-trimethyl-decanol | Increased | 2,6,10-trimethyl-decanol | Increased |
| 2,4-dimethyl-heptadecene | Increased | 2,4-dimethyl-heptadecene | Increased |
| Ethanol | Increased | Ethanol | Increased |
| 2-methylbutane | Increased | 2-methylbutane | Increased |
| 2-hexyl-1-decanol | Decreased | 2-hexyl-1-decanol | Decreased |
| Tetradecanal | Decreased | Tetradecanal | Decreased |

*MPM: meropenem, ND: no difference in abundance between MPM and saline treatment*

**Table 3. Differentially changing breath metabolites in mice infected with NDM-1*<sup>+</sup> vs. NDM-1- *Klebsiella pneumoniae***

| **Breath Volatile Metabolite** | **Klebsiella pneumoniae** | **Klebsiella pneumoniae** |
|-----------------|-----------------|-----------------|
| **Direction of change in abundance with MPM treatment** | **Microbe** | **Direction of change in abundance with MPM treatment** | **Microbe** |
| 2-methyl-butanone | Increased | 2-methyl-butanone | Increased |
| 2-methyl-2-propyl-1,3-propanediol | Increased | 2-methyl-2-propyl-1,3-propanediol | Increased |
| Ethanol | Increased | Ethanol | Increased |
| 2-methylbutane | Increased | 2-methylbutane | Increased |
| 2-hexyl-1-decanol | Decreased | 2-hexyl-1-decanol | Decreased |
| Tetradecanal | Decreased | Tetradecanal | Decreased |

**Disclosures. All authors:** No reported disclosures.
Background. To prevent the spread of drug-resistant bacteria, a rapid and accurate antimicrobial susceptibility test (AST) is necessary. Recently, morphokinetic microscopy approaches have been reported as a rapid AST method. However, these still require several hours to obtain a minimum inhibitory concentration (MIC). Adenosine triphosphate (ATP) luminescence has also been reported as a rapid AST method that can detect bacterial growth more rapidly than morphokinetic approaches, since ATP in bacteria increases prior to bacterial division. In this study, we designed a new machine learning-based algorithm that predicts MIC rapidly, using a dataset that contains ATP luminescence patterns and conventional MICs determined by turbidity. Essential agreement (EA) rates between rapid and conventional MIC were then evaluated.

Methods. Sixty-three strains of E. coli (ATCC 29522 and clinical isolates from Toyama University Hospital) were tested. Bacterial suspensions were diluted 500-fold in Mueller–Hinton broth from 0.5 McF solutions, and the final concentration of bacteria was 3 × 10^8 CFU/mL. The suspensions were dispensed into a 96-well microplate, which had 12 antimicrobials in two-fold dilution series, and the microplate was incubated at 35°C. At each measurement time point, the amount of ATP in a 10 µL aliquot from each well was evaluated by our original measurement system, which can sensitively detect ATP luminescence to a single bacterium. After 22 hours, MIC was determined conventionally by measuring turbidity. A rapid MIC for each bacterium was estimated by the algorithm based on the dataset consisting of the rest of the 62 strains (leave-one-out cross validation).

Results. Table 1 shows the EA rate for the 12 antimicrobials; EA rates > 90% were achieved for 7 antimicrobials in 2 hours and for 12 antimicrobials in 3 hours. In 6 hours, an average EA rate > 97% was achieved.

Conclusion. Using the dataset, our new machine learning-based algorithm predicted MIC rapidly within 2 hours with an EA rate > 90% for 7 antimicrobials. The rapid AST detected by the ATP luminescence method will contribute toward both appropriate antimicrobial treatment and reduction in medication charges. In the future, other species of bacteria will be evaluated by our ATP method.

Table 1. EA rate (%) by ATP luminescence and machine learning

| Antibiotic | Mean | 1:40 | 1:80 | 1:160 | 1:320 | 1:640 |
|------------|------|------|------|-------|-------|-------|
| 8-Bromo     | 99.7 | 99.2 | 98.8 | 95.2 | 95.2 | 100  |
| 1-Bromo     | 95.2 | 100  | 99.2 | 99.6 | 97.7 | 98.4 |
| 1-Chlorine  | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |
| 1-Iodo      | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |
| 1-Nitro     | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |
| 1-Phenyl    | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |
| 2-Chlorine  | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |
| 2-Phenyl    | 95.2 | 95.2 | 98.4 | 98.6 | 95.2 | 98.4 |

*All measurements were performed 3 times. ATP was calculated as 100% at 0.1 McF. APP: Adenosine triphosphate. P7: phenyl-piperazine-1-carboxylic acid. C7: chloramphenicol. C9: cloxacin. CEP: cephalosporin. CF: cefuroxime. CIP: ciprofloxacin. CSM: clindamycin. ETP: erythromycin. LEU: levofloxacin. MBC: minimum bactericidal concentration. MEPC: minimum eradication concentration. MMPM: minimum microbicidal concentration. HIP: hipromicin. S: essential agreement. Agreement within ± 1 dilution of conventional MIC

Disclosures. All authors: No reported disclosures.