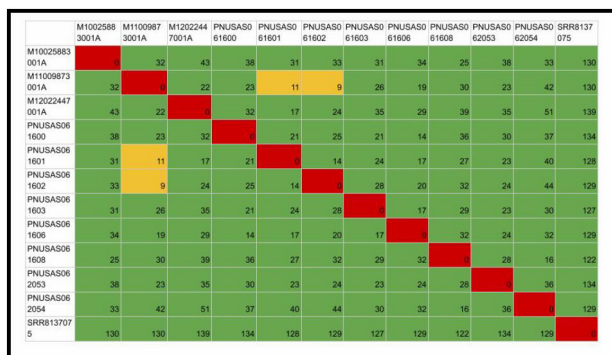was separated from those associated with human infections by 14 (n=1), 17 (n=1) and ≥20 (n=7).

Conclusion. WGS analysis revealed clinically relevant ESCs genes in closely related *S. Berta* isolates from human and animal sources. Presence of these genes in NTS highlights the need for enhanced One-Health surveillance and judicious use of antibiotics in humans and food-animal production.

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1437. Biochemical characterization of L1 and L2 β-lactamases from clinical isolates of Stenotrophomonas maltophilia

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Session: P-66. Resistance Mechanisms

Background. *Stenotrophomonas maltophilia* is a Gram-negative, non-fermenting opportunistic pathogen. Two β-lactamases provide intrinsic resistance to β-lactams: a class B Metallo- β-lactamase L1, and a class A serine β-lactamase (SβL) L2. Recently, we described novel variants of the L1 and L2 in a collection of clinical *S. maltophilia* isolates collected in the US, and showed through analyses of the amino acid sequences that L1 and L2 grouped into 4 (A-D, B, C, and E) and 2 (A and D) clades, respectively. We aimed to characterize the new L1 and L2 clinical variants biochemically.

Methods. Representative *bla*L1 and *bla*L2 genes from each of the identified clades were cloned into pBC-SK and pET24 vectors and transformed into *E. coli* DH10B and BL21 (DE3) cells, respectively. Minimal inhibitory concentrations (MICs) were determined using CLSI approved methods. Cell-based assays and biochemical characterization performed on purified enzymes, including circular dichroism (CD), thermal stability, and steady-state kinetics assays, were performed.

Results. Susceptibility testing using DH10-B *E. coli* strains expressing the L1 and L2 variants are shown in Table 1. Remarkably, while all L1 variants confer the same level of resistance to carbapenems, L2B conferred higher MICs to 3rd gen cephalosporins and aztreonam than L2D. Kinetics assays confirmed differences in the $k$cat of both enzymes to ceftazidime (32s-1 for L2B vs. 7s-1 for L2D) and avibactam inhibition constant Ki (1.7 μM for L2B vs. 4.5 μM for L2D). Structurally, L2B and L2D present distinctive CD spectra and thermal stabilities (ΔTm 5°C).

Table 1

Table 1. Minimum inhibitory concentrations of *E. coli* pBC SK (+) producing L1 and L2 variants.

	TAZ	TAX	FEP	PIP	ATM	TZP	TIM	SAM	CZA	IPM	MEM
pBCSK+	0.5	0.125	0.06	4	0.25	≤2	≤16	≤16	≤0.5	0.5	≤0.125
L1a	512	64	4	2048	0.25	2048	>4096	4096	>128	32	32
L1b	512	64	4	2048	0.25	2048	>4096	4096	>128	16	16
L1c	512	128	4	2048	0.25	1024	>4096	4096	>128	16	16
L1d	512	64	4	1024	0.25	1024	>4096	4096	>128	8	16
L1e	16	16	0.5	1024	0.25	1024	>4096	4096	16	32	32
L2b	128	32	2	512	2048	512	256	64	0.5	0.5	0.125
L2d	8	8	2	512	512	256	128	128	0.25	0.5	0.125

TAZ: ceftazidime; TAX: cefotaxime; FEP: cefepime; PIP: piperacillin; ATM: aztreonam; TZP: piperacillin tazobactam; TIM: ticarcillin clavulanate; SAM: ampicillin sulbactam; IPM: imipenem; MEM: meropenem. Values in red represent ≥ 2 folds difference in the MIC value.

Conclusion. As opposed to the L2 variants, our results suggest that the L1 variants may not be functionally nor structurally different. Differences between L2B and L2D might have arisen due to the use of cephalosporins and SβL inhibitors. Further experiments are on the way to determine the structural basis of these observations and the implication of these for the design of novel β-lactamase inhibitors.

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1438. Dissecting the Multifaceted Nature of Antibiotic Resistance in Clinical Isolates of Neisseria gonorrhoeae by Natural Transformation

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Session: P-66. Resistance Mechanisms

Background. *Neisseria gonorrhoeae* (NG) causes the sexually transmitted disease gonorrhea. It has developed resistance to every antibiotic introduced for gonorrhea treatment such that NG clinical isolates with multidrug resistance (MDR) are increasingly common. We hypothesize that natural transformation could be used to transfer genetic determinants of antibiotic resistance from drug-resistant NG clinical isolates without pre-knowledge of the genetic determinants to a new background under antibiotic selection to generate isogenic transformants for further characterization.

Methods. Natural transformation, PCR amplification and DNA sequencing, and antibiotic susceptibility testing were used in the studies.

Results. We have validated the hypothesis using genomic DNA from an MDR including ciprofloxacin-resistant NG clinical isolate as a donor and a ciprofloxacin-susceptible NG isolate as a recipient under the selective pressure of ciprofloxacin. This led to a series of transformants that contain single or multiple coding resistance determinants being generated depending on the resistance levels and transformation frequencies. Antibiotic susceptibility testing and genetic characterization of the transformants allowed us to (i) identify ciprofloxacin resistance determinants including efflux mutation *mtrR*$_{-79}$ and target mutations GyrA S91F D95G and ParC D86N, (ii) quantify the contribution of each genetic determinant responsible for the ciprofloxacin resistance and (iii) regenerate the ciprofloxacin resistance phenotype of the donor isolate to detect multiple paths of possible resistance development for ciprofloxacin. Furthermore, we also validated the hypothesis with a novel antibiotic gepotidacin to identify pre-existing genetic determinants contributing to varying susceptibility to this antibiotic. Finally, we envision that, along with whole genome sequencing, natural transformation could be used to identify and quantify novel genetic resistance determinants to current or novel antibiotics in drug-resistant NG clinical isolates.

Conclusion. We demonstrated the utility of natural transformation in dissecting the multifaceted nature of antibiotic resistance in NG clinical isolates.

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1439. High Rates of Drug Resistance in Escherichia coli from a Pilot Antimicrobial Resistance Surveillance System in Cambodia

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Session: P-66. Resistance Mechanisms

Background. Antimicrobial resistance (AMR) is a major and growing global public health problem. The Cambodia Ministry of Health established a pilot laboratory-based AMR surveillance system for blood specimens in 2017. The objective of this study is to characterize AMR among pathogenic isolates from blood samples.

Methods. A retrospective analysis was performed using one year of data from a pilot AMR Surveillance system in Cambodia. Four blood culture pathogens were included: *Escherichia coli, Klebsiella pneumoniae, Salmonella Typhi /Salmonella Paratyphi A* and *Staphylococcus aureus*. Blood culture isolates that were referred from eleven sentinel sites were analyzed at the National Public Health Laboratory for identification. Antibiotic susceptibility testing (AST) was done using disk diffusion, minimum inhibitory concentration method following Clinical Laboratory Standard Institute (CLSI) guidelines.

Results. Among 214 pathogenic isolates from blood samples, *E.coli* was the most common (56.1%), followed by *Salmonella Typhi/Salmonella Paratyphi* A (18.7%), *Staphylococcus aureus* (13.5%), and *Klebsiella pneumoniae* (11.7%). Methicillin Resistance *Staphylococcus aureus* (MRSA) was detected in half of the isolates. E.coli was resistant to ampicillin (94.4%), trimethoprim-sulfamethoxazole (84.5%), and ceftriaxone (79.2%). *Salmonella Typhi* was resistant to ampicillin (73.3%) and trimethoprim-sulfamethoxazole (60.0%) and *Salmonella Paratyphi A* were resistant to fluoroquinolones (91.7%). For last resort antibiotics, *E.coli* was resistant to carbapenem groups (3.2% for imipenem, 4.9% for meropenem, and 5.0% for ertapenem). *Klebsiella pneumoniae* was not resistant to any groups.

Conclusion. *E.coli* was found at high rates in blood samples and was resistant to common antibiotics used in Cambodia. These pilot data show the importance of establishing a national AMR surveillance system in Cambodia to monitor AMR trends following GLASS guidelines.

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