In Silico Evaluation of Ceftriaxone Susceptibility Prediction among *Enterobacteriaceae* by Two Rapid Blood Culture Diagnostic Tests

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SATURDAY - AAR-728

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AMENDED ABSTRACT

BACKGROUND: The use of rapid diagnostic tests (RDTs) for blood cultures has become standard of care in the United States to inform early antimicrobial optimization. However, the relative ability of RDT systems to identify select phenotypes across different geographic epidemiology is unclear.
MATERIALS AND METHODS: This study evaluated, *in silico*, the relative ability of a genotypic and phenotypic RDT, to identify beta-lactam susceptibility in *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis*, using a previously published database that documented epidemiology of these organisms in 72 U.S. hospitals in 2012. The relative ability of the FDA-cleared genotypic and phenotypic RDTs to identify beta-lactam not susceptible phenotypes was modeled, using incidence rates of resistance mechanisms to beta-lactams seen across U.S. census regions. The genotypic test detects the presence of six beta-lactamase genes, while the phenotypic test specifically determines antimicrobial agent MICs. Analytical performance characteristics (sensitivity) of each approach were evaluated and extrapolated as the expected performance per 100 tests.
RESULTS: Overall, presence of CTX-M, KPC and/or NDM genes was 81% (range, 57 – 87%) sensitive for prediction of ceftriaxone, ceftazidime and aztreonam resistance and 73% (range, 25-90%) sensitive for the detection of piperacillin-tazobactam resistance. Sensitivity of KPC or NDM to predict imipenem or meropenem resistance was 94.3% overall, and for meropenem ranged from 70 – 100% across United States census regions. Very major errors (i.e., false prediction of ceftriaxone susceptibility based on absence of gene or MIC), ranged from 1.0 – 10.3% and 0.2 –1.9% for the genotypic and phenotypic RDT, respectively.
CONCLUSION: Institutions that use genotypic RDTs to inform therapeutic de-escalation decisions should be aware of the incidence-base performance across both U.S. geographies, and in different patient populations. Knowledge of the isolate's MIC as soon as possible may significantly aid in the management of these complicated cases.

OBJECTIVES

- The use of rapid diagnostic tests (RDTs) for blood cultures to inform early antimicrobial optimization has become the new standard of care in the U.S.
- With publication of the MERINO trial, knowing whether an isolate is ceftriaxone (CRO) not susceptible is imperative, to inform therapy
- At present, in the U.S., two RDTs are FDA-cleared that might be used to determine an isolate's CRO susceptibility, directly from positive blood cultures. These are a molecular test, (Verigene® GN, Luminex®), which detects the presence of CTX-M, KPC, NDM, IMP, VIM and Oxa-48-like beta-lactamases, and a phenotypic test (Accelerate PhenoTest™ BC kit, Accelerate Diagnostics, Inc.), which specifically determines CRO, ceftazidime (CAZ), aztreonam (ATM), piperacillin-tazobactam (TZP) and meropenem (MEM) MICs, among those for other antimicrobial agents
- The intention of this study was to evaluate the relative rates of CRO, CAZ, ATM, and TZP not susceptible *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca* and *Proteus mirabilis* in the U.S. that might be predicted using a genotype evaluation. Prediction of imipenem (IPM) and MEM resistance was also evaluated by the genotype approach

METHODS

- Raw data (per-isolate basis) was obtained from JMI Laboratories for their surveillance study of ESBL, AmpC and carbapenemase enzyme prevalence across the U.S. (1)
- This survey evaluated 5739 isolates of *E. coli*, *Klebsiella* spp and *Proteus mirabilis* collected from 72 US hospitals in the 2012 calendar year
- Isolates that met CLSI screening criteria for the presence of an ESBL (i.e., MIC >1 µg/mL to CRO, CAZ or ATM) by CLSI reference broth microdilution (2) were tested for the presence of ESBL (CTX-M, SHV and TEM), AmpC (ACC, ACT/MIR, CMYI/MOX, CMYII, DHA and/or FOX), KPC and NDM beta-lactamases using the Check-MDR CT101 kit (Check-points, Wageningen, Netherlands)
- In total, 747 isolates met these criteria, & comprise the dataset for this evaluation.
- Analytical performance characteristics (sensitivity) of a genotypic approach for detection of CTX-M, KPC and NDM were evaluated
- As some have endorsed use of CRO, CAZ or ATM for *Klebsiella* or *E. coli* that test negative for CTX-M or carbapenemase (3), isolates that did not harbor these genes but were not susceptible to these antimicrobials were classified as very major errors (VME)
- Isolates that were positive for CTX-M, NDM and/or KPC were considered to be in categorical agreement with a not susceptible phenotype to these antimicrobials
- MEM and IPM phenotypic results were compared to NDM and KPC results
- Performance was extrapolated across a 5% to 50% resistance rate
- This was done by calculating the number of resistant isolates that would be predicted to be susceptible in a hypothetical sampling of 100 isolates, at each prevalence rate.
- Upper & lower confidence limits of a 95% exact binomial confidence interval were calculated. Average national rates & observed rates by census region were utilized

RESULTS

- Composite performance of a genotypic approach is shown in Table 1 for CRO, CAZ, ATM and TZP
- Overall, 80% of isolates that were CRO, CAZ or ATM not susceptible were POS for ≥1 of CTX-M, KPC or NDM, indicating a 20% VME rate, if a NEG result was used to predict susceptibility
- Among the non-CTX-M resistance mechanisms that accounted for resistance to these antimicrobials, SHV ESBLs were the most common, followed by AmpC
- Use of KPC or NDM to predict resistance to IPM and MEM are shown in Table 2
- Sensitivity to detect imipenem resistance/not susceptible MICs was 98.0%/88.9% and meropenem resistance/not susceptible MICs was 94.8%/92.1%
- The ability of the genotype to detect resistance / not susceptible MICs to either IPM or MEM was 94.3 / 86.0%
- The specific MICs to CRO, CAZ, IPM, and MEM were evaluated for the isolates that were not susceptible to these antimicrobials (Figure 1)
- The performance of the genotypic method across geographic areas was evaluated (Table 3)
- The number of very major errors associated with using the genotypic vs. phenotypic method to predict CRO susceptibility was evaluated across different prevalence of resistance to these antimicrobials (Table 4)
- VME (i.e., false prediction of ceftriaxone susceptibility based on absence of gene or MIC), ranged from 1.0 – 10.3% and 0.2 –1.9% for the genotypic and phenotypic RDT, respectively

Table 1. Utility of a genotypic result of CTX-M/KPC/NDM to predict CRO, CAZ, ATM, and TZP results

	NEG for CTX-M, KPC,& NDM	POS for CTX-M, NDM &/or KPC	% VME	If negative for CTX-M, KPC, & NDM, presence of other BLA, %			
				AmpC	TEM	SHV	neg
CRO-NS	145	557	20.7	33.8	0.6	36.6	29.0
CAZ-NS	109	436	20.0	35.7	0.9	45.0	18.3
TZP-NS	93	250	27.1	32.3	0.3	17.2	47.3
ATM-NS	139	533	20.7	28.8	0.7	36.7	33.8

Table 2. Utility of a genotypic result of KPC/NDM to predict IPM/MEM results

	NEG for NDM &/or KPC	POS for NDM &/or KPC	% VME
IPM-R	3	150	2.96
MEM-R	8	145	5.23
IPM-NS	19	152	11.1
MEM-NS	13	152	7.88

Table 3. Predicted performance of genotypic approach* across US geographies

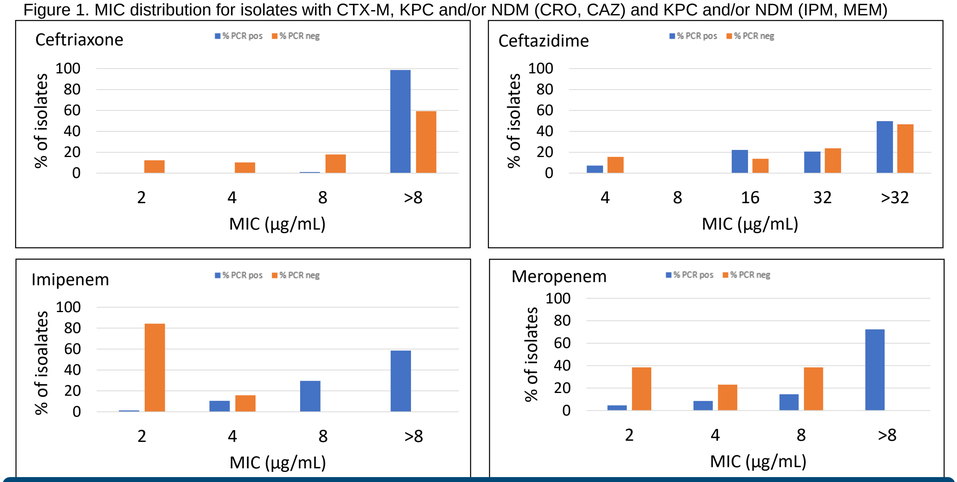
Census Region	CRO		MEM		TZP	
	NS (n)	% VME	NS (n)	% VME	NS (n)	% VME
1. New England	42	42.9	4	25.0	23	60.9
2. Mid-Atlantic	255	12.2	116	3.5	166	9.6
3. East North Central	80	25.0	13	7.7	31	41.9
4. West North Central	27	33.3	0	N/A	8	75.0
5. South Atlantic	38	29.0	5	0.0	21	47.6
6. East South Central	51	27.5	1	0.0	12	66.7
7. West South Central	131	18.3	23	30.4	56	23.2
8. Mountain	23	26.1	2	0.0	7	57.1
9. Pacific	55	21.8	1	0.0	19	47.4
Overall	702	20.7	165	7.3	343	27.1

*genotypic approach includes detection of CTX-M, KPC and/or NDM for CRO/TZP, & KPC or NDM for MEM

Table 4. Modeled performance of a genotypic and phenotypic approach to predict CRO not susceptible *E. coli*, *Klebsiella* spp & *Proteus mirabilis* across different resistance rates

% CRO R	Genotypic		Phenotypic	
	VME (n)	95% CI	VME (n)	95% CI
5	1.0	0.2-1.2	0.2	0.1-0.5
10	2.1	0.5-2.4	0.4	0.1-0.9
15	3.1	0.7-3.6	0.6	0.2-1.4
20	4.1	0.9-4.8	0.7	0.3-1.8
25	5.2	1.2-5.6	0.9	0.4-2.3
30	6.2	1.4-7.1	1.1	0.4-2.8
35	7.2	1.6-8.3	1.3	0.5-3.2
40	8.3	1.9-9.5	1.5	0.6-3.7
45	9.3	2.1-10.7	1.7	0.7-4.1
50	10.3	2.3-11.9	1.9	0.7-4.6

Figure 1. MIC distribution for isolates with CTX-M, KPC and/or NDM (CRO, CAZ) and KPC and/or NDM (IPM, MEM)



CONCLUSIONS

- Institutions that use genotypic RDTs to inform therapeutic de-escalation decisions should be aware of the incidence-base performance across both U.S. geographies, and in different patient populations
- Knowledge of isolate's MIC as soon as possible may aid in the management of these complicated cases

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