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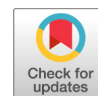

Evaluation of the Vitek 2, Phoenix, and MicroScan for Antimicrobial Susceptibility Testing of *Stenotrophomonas maltophilia*

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ABSTRACT *Stenotrophomonas maltophilia* causes high-mortality infections in immunocompromised hosts with limited therapeutic options. Many U.S. laboratories rely on commercial automated antimicrobial susceptibility tests (cASTs) and use CLSI breakpoints (BPs) for *S. maltophilia*. However, contemporary data on these systems are lacking. We assessed performance of Vitek 2, MicroScan WalkAway, and Phoenix relative to that of reference broth microdilution for trimethoprim-sulfamethoxazole (SXT), levofloxacin (LEV), minocycline (MIN), and ceftazidime (CAZ) with 109 *S. maltophilia* bloodstream isolates. Using CLSI breakpoints, categorical agreement (CA) was below 90% on all systems and drugs, with the exception of SXT by MicroScan (98.1%) and Phoenix (98.1%) and MIN by MicroScan (100%) and Phoenix (99.1%). For SXT, Vitek 2 yielded a 77.1% CA. LEV and CAZ CA ranged from 67% to 85%. Very major errors (VME) were >3% for SXT (MicroScan, Phoenix), LEV (MicroScan), and CAZ (all systems). Major errors (ME) were >3% for SXT (Vitek 2), LEV (Phoenix), and CAZ (MicroScan, Phoenix). Minor errors were >10% for CAZ and LEV on all systems. Data were analyzed with EUCAST pharmacokinetic/pharmacodynamic CAZ, LEV, ciprofloxacin (CIP), and tigecycline (TGC) breakpoints when possible. CA was <90% for all. VME were >3% for CAZ (all systems), LEV (MicroScan), and TGC (Vitek 2), and ME were >3% for LEV (MicroScan), CAZ (all systems), ciprofloxacin (Vitek 2 and MicroScan), and TGC (Vitek 2, Phoenix). Minor errors (MI) were >10% for all agents and systems, by EUCAST breakpoints with an intermediate category (LEV, CAZ, CIP). Laboratories should use caution with cASTs for *S. maltophilia*, as a high rate of errors may be observed.

KEYWORDS *Stenotrophomonas*, antimicrobial activity, antimicrobial agents, assay standardization, automation, bloodstream infections, breakpoints, diagnostics, immunocompromised hosts, susceptibility testing

Healthcare-associated infections caused by multidrug-resistant (MDR) organisms are a serious clinical challenge, often leaving clinicians with few to no treatment options. Of particular challenge is the management of infections caused by *Stenotrophomonas maltophilia*, an organism which is intrinsically resistant to a variety of antimicrobial classes, including penicillins, most cephalosporins, β-lactam combination agents, carbapenems, and aminoglycosides (1). Crude mortality rates for bacteremia caused by *S. maltophilia* range from

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30 to 70%, with those patients receiving inappropriate antimicrobial therapy having the highest risk of mortality (2, 3).

A majority of clinical laboratories in the United States use commercial automated antimicrobial susceptibility test systems (cASTs), including the Vitek 2 (bioMérieux, Durham NC), Phoenix (BD Biosciences, Sparks, MD), and MicroScan WalkAway (Beckman Coulter, Sacramento, CA) to perform susceptibility testing. These systems are associated with simplified workflow and reduced time to results compared to traditional testing methods. However, three primary hurdles complicate the use of cASTs for *S. maltophilia*: (i) the U.S. FDA recognizes only ceftazidime (CAZ) clinical breakpoints (BPs) for *S. maltophilia*, (ii) the 2009 Food and Drug Administration (FDA) class II special controls guidance document requires manufacturers to use FDA BPs alone for the clearance of new/updated cASTs, and (iii) there are limited contemporary data which demonstrate the performance of cASTs for *S. maltophilia* (4). No cAST has been reviewed by the U.S. FDA for its ability to accurately perform AST for *S. maltophilia* since before 2009 (5). Furthermore, if a performance issue were to be recognized for *S. maltophilia* testing, there is no regulatory pathway by which a manufacturer might update their cASTs to improve performance.

Trimethoprim-sulfamethoxazole (SXT) is the drug of choice for treatment of *S. maltophilia* and retains good activity against isolates recovered in surveillance studies from 5 years ago (1). However, SXT is associated with myelosuppression, which precludes its use for some patients, including those with hematological malignancies, which are a major risk factor for infection by *S. maltophilia*. As such, testing of additional agents like levofloxacin (LEV), minocycline (MIN), CAZ, ciprofloxacin (CIP), and tigecycline (TGC) is often performed. We recently reported that disk and gradient diffusion test performance for *S. maltophilia* was suboptimal for many of these agents (6). The present study evaluates, using the same collection of isolates, the performance of three cAST systems for testing of *S. maltophilia*, Vitek 2 AST-GN69 cards, Phoenix NMIC Gram-negative panel, and MicroScan WalkAway Neg MIC 53 panel.

MATERIALS AND METHODS

Bacterial strains. A total of 110 *S. maltophilia* strains were recovered from clinical blood cultures spanning 2015 to 2019 with the following susceptibility patterns selected from clinical laboratory susceptibility results: ~10% resistant to SXT, ~30% resistant to CAZ, and ~20% resistant to LEV. The majority of the isolates came from MD Anderson Cancer Center, followed by University of California, Los Angeles and Children's Hospital of Los Angeles (6). Isolates were collected by a central laboratory and subcultured, and frozen stocks were prepared using the same bacterial suspension and distributed to the 4 testing laboratories. A total of 109 isolates were included in this study, with 1 removed due to the lack of growth by broth microdilution (BMD). Prior to testing, frozen isolates were subcultured twice on tryptic soy agar with 5% sheep blood (BAP; BD, Sparks, MD). Quality control (QC) strains tested with each run included *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC was within range.

Antimicrobial susceptibility testing. All isolates were tested at four separate laboratories, each performing one of the four distinct AST methodologies, BMD, Vitek 2, Phoenix, and MicroScan. An inoculum matching a 0.5 McFarland standard was prepared using 3 to 5 isolated colonies from a single plate. BMD MIC testing was performed according to CLSI standards using custom BMD panels prepared by Accelerate Diagnostics (7). Vitek 2, MicroScan, and Phoenix testing was performed with the AST-GN69 cards, NMIC Gram-negative panel, and Neg MIC 53 panel, respectively, according to manufacturer's instructions. BMD panels and plates were incubated in ambient air at 35°C ± 2°C and read after 20 to 24 h of incubation, and the automated systems were operated according to manufacturer's instructions.

Data analysis. Susceptibility results were compared to assess performance of the cAST assays in relation to BMD. Categorical agreement (CA), essential agreement (EA), major errors (ME), very major errors (VME), and minor errors (MI) were evaluated. EA was defined as agreement within ±1 2-fold dilution of the method under evaluation with BMD. MICs that were off-scale were assumed to be in EA, unless BMD results were on-scale and more than 1 dilution away from the highest or lowest concentration tested. For example, an MIC of ≤1 μg/ml by the cAST but 4 μg/ml by BMD would be scored as out of EA, whereas an MIC of ≤1 μg/ml by the cAST but 2 μg/ml by BMD would be scored as in EA. CA was defined as the agreement of interpretative results between the method under evaluation and BMD using the CLSI or EUCAST pharmacokinetic/pharmacodynamic (PK/PD) breakpoints which are non-species related, as appropriate. Discrepancies between the method under evaluation and BMD were categorized as follows: VME, false-susceptible result by the cAST and resistant by BMD; ME, false-resistant result by the cAST and susceptible by BMD; MI, a discrepancy between the test and reference methods involving an intermediate category. Acceptable performance was defined as a CA of >89.9% and an EA of

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TABLE 1 MIC breakpoints used in this study[a]

Antimicrobial (breakpoint source)	No. of MIC breakpoints (μg/ml)		
	S	I*	R
Trimethoprim-sulfamethoxazole (CLSI M100 S30)	≤2		≥4
Levofloxacin (CLSI M100 S30)	≤2	4	≥8
Minocycline (CLSI M100 S30)	≤4	8	≥16
Ceftazidime (CLSI M100 S30)	≤8	16	≥32
Ciprofloxacin (EUCAST PK/PD)	≤0.25	0.5	>0.5
Ceftazidime (EUCAST PK/PD)	≤4	8	>8
Levofloxacin (EUCAST PK/PD)	≤0.5	1	>1
Tigecycline (EUCAST PK/PD)	≤0.5		>0.5

[a]S, susceptible; I*, intermediate (CLSI definition) or susceptible with increased exposure (EUCAST definition); R, resistant. PK/PD refers to pharmacokinetic/pharmacodynamic breakpoints that are not species-specific.

>89.9%, with VME and ME of <3% and MI of <10% (8). Breakpoints used to categorize isolates are listed in Table 1.

RESULTS

Susceptibility distribution of isolates by BMD and across cASTs. Of the 110 *S. maltophilia* bloodstream isolates tested, 1 isolate was eliminated due to repeat growth failures by reference broth microdilution (BMD). Additionally, one isolate resulted in repeat growth failures on the MicroScan, as did 2 isolates on the Phoenix, resulting in 109 isolates tested by BMD and Vitek 2, 108 by MicroScan, and 107 by Phoenix. Six antimicrobials (SXT, LEV, MIN, CAZ, CIP, and TGC) with clinical utility in the treatment of *S. maltophilia* infections were evaluated, as available on each system.

Overall, significant agent-dependent variability in susceptibility profiles was noted for this collection of isolates across the cASTs platforms when CLSI breakpoints were applied (Fig. 1; Table 1). For SXT, Vitek 2 resulted in susceptibility (68%) significantly lower than that of BMD (91%), MicroScan (94%), and Phoenix (94%). In contrast, Vitek 2 overcalled susceptibility (76% susceptible) for CAZ relative to BMD (50%), MicroScan (47%), and Phoenix (49%) (Fig. 1A). LEV and MIN susceptibility rates were similar across systems.

LEV, CIP, CAZ, and TGC were evaluated by EUCAST PK/PD breakpoints which are non-species related (Table 1) in the absence of other EUCAST guidelines for these agents and *S. maltophilia*. EUCAST SXT *S. maltophilia*-specific breakpoints could not be evaluated on the automated systems, as the lowest SXT concentration tested (1 μg/ml) was above the EUCAST susceptible breakpoint (≤0.001 μg/ml) (Table 1). Overall, the

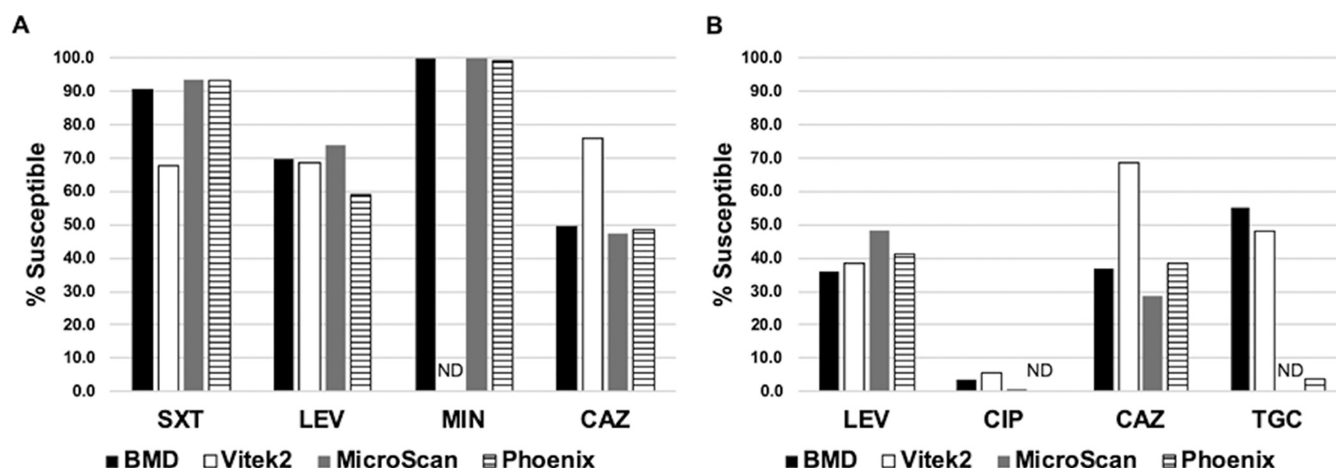

FIG 1 Categorization of *S. maltophilia* clinical isolates across AST methodologies. (A) MICs categorized by CLSI breakpoints. (B) MICs categorized by EUCAST breakpoints. SXT was not evaluable by EUCAST breakpoints due to the very low susceptible breakpoint of ≤0.0001 μg/ml; all other EUCAST breakpoints are generic PK/PD breakpoints. ND, no data.

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TABLE 2 Performance of Vitek 2, MicroScan, and Phoenix compared to BMD for *S. maltophilia* bloodstream isolates with CLSI breakpoints (M100 30th edition)[a]

Test system and antimicrobial	EA (%)	CA (%)	No. of VME (%)	No. of ME (%)	No. of MI (%)
Vitek 2					
Trimethorpim-sulfamethoxazole	69/109 (63.3)	84/109 (77.1)	0/10 (0.0)	25/99 (25.3)	NA
Levofloxacin	100/109 (91.7)	93/109 (85.3)	0/22 (0.0)	2/76 (2.6)	13/109 (12)
Ceftazidime	55/108 (50.9)	73/108 (67.6)	19/43 (44.2)	0/54 (0.0)	16/108 (14.8)
MicroScan					
Trimethorpim-sulfamethoxazole	83/108 (76.9)	106/108 (98.1)	2/9 (22.2)	0/99 (0.0)	NA
Levofloxacin	94/108 (87.0)	88/108 (81.5)	2/22 (9.1)	2/75 (2.7)	16/108 (14.8)
Ceftazidime	83/108 (76.9)	75/108 (69.4)	4/43 (9.3)	7/54 (13.0)	22/108 (20.4)
Minocycline	108/108 (100)	108/108 (100)	NA	0/108 (0.0)	0/108 (0.0)
Phoenix					
Trimethorpim-sulfamethoxazole	85/107 (79.4)	105/107 (98.1)	2/9 (22.2)	0/98 (0.0)	NA
Levofloxacin	92/107 (86.0)	85/107 (79.4)	0/21 (0.0)	3/75 (4.0)	19/107 (17.8)
Ceftazidime	72/107 (67.3)	76/107 (71.0)	7/43 (16.3)	11/53 (20.8)	13/107 (12.1)
Minocycline	100/107 (93.5)	106/107 (99.1)	NA	0/107 (0.0)	1/107 (0.9)

[a]Categorical agreement (CA), essential agreement (EA), very major errors (VME), major errors (ME), and minor errors (MI) calculated. NA, not applicable due to the absence of an intermediate interpretive category.

EUCAST BPs resulted in susceptibility rates significantly lower than those of CLSI. Over half of the isolates in our study were categorized as not susceptible by all methods using EUCAST breakpoints, with the exceptions of CAZ on the Vitek 2 (69% susceptible) and TGC by BMD (55% susceptible) (Fig. 1B).

Vitek 2 performance versus BMD performance. Using CLSI M100 S30 breakpoints, Vitek 2 did not have acceptable performance for any agent tested. A categorical agreement (CA) of 77.1% and an essential agreement (EA) of 63.3% were observed for SXT with 0 very major errors (VME) and 25 major errors (ME, 25%) (Table 2). Of the 25 ME, 9 isolates had a Vitek 2 MIC of $>16\,\mu$g/ml, whereas BMD MICs were $0.5\,\mu$g/ml ($n=1$), $1\,\mu$g/ml ($n=1$), or $2\,\mu$g/ml ($n=7$). For LEV, 85.3% CA and 91.7% EA overall were observed with no VME, 2 ME (2.6%), and 13 MI (12%). The Vitek 2 reported an MIC of $>8\,\mu$g/ml for both ME, whereas BMD MICs of $2\,\mu$g/ml and $0.5\,\mu$g/ml were obtained. Of the 13 MI, 10 were in EA with BMD, of which Vitek 2 resulted in a more susceptible interpretation (i.e., susceptible versus intermediate or intermediate versus resistant) for 9. For CAZ, one isolate did not yield a result on the Vitek 2. Among the remaining 108 isolates, 67.6% CA and 50.9% EA compared to BMD were observed. Nineteen VME (44.2%), 0 ME, and 16 MI (14.8%) were observed. Of the 19 VME, 7 had BMD MIC of $32\,\mu$g/ml, 9 of $64\,\mu$g/ml, and 3 of $\geq 128\,\mu$g/ml. For the latter 3 isolates, one isolate each had a Vitek 2 CAZ MIC of $<1\,\mu$g/ml, $2\,\mu$g/ml, and $8\,\mu$g/ml. Among the 16 CAZ MI, of which only 1 was in EA, 7 were categorized as resistant by BMD and intermediate by Vitek 2 and 9 as intermediate by BMD but susceptible by Vitek 2. MIN was not present on the Vitek 2 AST panel and thus was not evaluated.

EUCAST PK/PD breakpoints were applied to the MICs; none of the agents met acceptable performance criteria, and all but CAZ had worse performance relative to the CLSI breakpoints. Vitek 2 had a CA of 78.9%, 72.5%, 78.9%, and 88.0% for LEV, CAZ, CIP, and TGC, respectively (Table 3). For LEV, Vitek 2 yielded 3 VME, 1 ME, and 19 MI. Among the MI, all 19 were intermediate by BMD but susceptible by Vitek 2. Vitek 2 reported an MIC of $0.5\,\mu$g/ml for all 3 VME, of which 2 had a BMD MIC of $2\,\mu$g/ml and 1 had an MIC of $4\,\mu$g/ml. For CIP, Vitek 2 yielded 2 VME, 1 ME, and 20 MI, of which 15 were in EA (Table 3). Of the 20 MI, Vitek 2 resulted in a more susceptible interpretation for 14. For CAZ by EUCAST PK/PD breakpoints, Vitek 2 yielded 23 VME, 0 ME, and 21 MI (Table 3). Of the 21 MI, 19 isolates were reported as more resistant by BMD than by Vitek 2.

MicroScan WalkAway performance versus BMD performance. With CLSI M100 breakpoints, MicroScan had acceptable performance only for MIN, with CA and EA of

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TABLE 3 Overall performance of Vitek 2, MicroScan, and Phoenix platforms compared to BMD for *S. maltophilia* bloodstream isolates with PK/PD breakpoints[a]

Test system and antimicrobial	Categorical agreement (%)	No. of VME (%)	No. of ME (%)	No. of MI (%)
Vitek 2				
Levofloxacin	86/109 (78.9)	3/51 (2.9)	1/39 (2.6)	19/109 (17.4)
Ciprofloxacin	86/109 (78.9)	2/92 (2.2)	1/4 (25.0)	20/109 (18.3)
Ceftazidime	79/109 (72.5)	23/55 (41.8)	0/40 (0.0)	21/109 (19.3)
Tigecycline	88/109 (88.0)	7/49 (14.3)	14/59 (23.7)	NA
MicroScan				
Levofloxacin	84/108 (84.0)	6/50 (12.0)	2/39 (5.1)	16/108 (14.8)
Ciprofloxacin	95/108 (88.0)	0/91 (0.0)	2/4 (50.0)	12/108 (11.1)
Ceftazidime	78/108 (72.2)	6/54 (11.1)	8/40 (20.0)	16/108 (14.8)
Phoenix				
Ceftazidime	67/107 (62.6)	9/54 (16.7)	8/39 (20.5)	23/107 (21.5)
Tigecycline	51/107 (47.7)	1/49 (2.0)	55/58 (94.8)	NA

[a]Categorical agreement (CA), very major errors (VME), major errors (ME), and minor errors (MI) calculated. Note: MIN is absent on the Vitek 2 card and lacks PK/PD breakpoints. CIP is absent on the Phoenix card. TGC was uninterpretable on the MicroScan since the breakpoint (0.5 μg/ml) is below the lowest reported value ($\leq$2 μg/ml). NA, not applicable due to the absence of an intermediate interpretive category.

100% and no errors. SXT had 98.1% CA, 76.9% EA, 0 ME, 0 MI, and 2 VME (22.2%). The two SXT VME were for isolates with BMD MICs of $>$16 μg/ml and 4 μg/ml and MicroScan MICs of 2 μg/ml and $\leq$0.5 μg/ml, respectively. Similarly, MicroScan LEV performance was suboptimal, with 81.5% CA, 87.0% EA, 2 VME (9.1%), 2 ME (2.7%), and 16 MI (14.8%). Of the 16 LEV MI, 15 were more susceptible than the BMD result. CAZ was associated with 69.4% CA, 76.9% EA, 4 VME (9.3%), 7 ME (13.0%), and 22 MI (20.4%) (Table 2). One of the 4 VME was for an isolate with a BMD MIC of 128 μg/ml but MicroScan MIC of 4 μg/ml. Of the ME, one was for an isolate with an MIC of 1 μg/ml and the other for an isolate with an MIC of 2 μg/ml by BMD but MICs of $>$16 μg/ml by MicroScan. The remaining 5 ME were for isolates with BMD MIC of 4 to 8 μg/ml and MicroScan MICs of $>$16 μg/ml. Out of 22 MI, 15 were for isolates with MIC values in EA between the MicroScan and BMD and 14 resulted in an interpretation more susceptible by MicroScan than by BMD.

When EUCAST PK/PD BPs were applied to the MICs, CA of 84.0%, 88.0%, and 72.2% were observed for LEV, CIP, and CAZ, respectively (Table 3). For LEV, MicroScan yielded 6 VME, 2 ME, and 16 MI. All VME were isolates with MICs ranging from 2 to 4 μg/ml by BMD (susceptible by CLSI breakpoints) that the MicroScan reported as $\leq$0.5 μg/ml. Conversely, the two VME observed by CLSI breakpoints were resistant by both BMD and MicroScan using EUCAST breakpoints. Of the 16 MI, 15 were more susceptible by MicroScan than by BMD. For CIP, MicroScan yielded 2 ME and 12 MI, of which 1 was more susceptible by MicroScan than BMD. Both ME had an MIC of 0.5 μg/ml by BMD but MicroScan MICs of 1 and 2 μg/ml. For CAZ, MicroScan yielded 6 VME, 8 ME, and 16 MI. Four of the MI were more susceptible by MicroScan than by BMD, for CAZ and EUCAST breakpoints. We did not evaluate TGC since the MicroScan generates an unevaluable MIC of $<$2 μg/ml, which is significantly above the EUCAST PK/PD breakpoint ($\leq$0.5 μg/ml).

Phoenix performance versus BMD performance. Similar to the MicroScan, Phoenix only showed acceptable performance for MIN with CA and EA of 99% and 1 MI (1%) (Table 2). SXT had 98.1% CA, 79.4% EA, 2 VME, and 0 ME. One of the two VME was for an isolate that also resulted in a VME by the MicroScan, due to a BMD MIC of $>$16 μg/ml, but MicroScan and Phoenix MIC of 2 μg/ml (Table 2). LEV had 79.4% CA, 86% EA, 0 VME, 3 ME (4.0%), and 19 MI (17.8%) (Table 2). Among the MI, 7 were in EA with BMD. Five MI were more susceptible by Phoenix than by BMD. CAZ had 71.0% CA, 67.3% EA, 7 VME (16.3%), 11 ME (20.8%), and 13 MI (12.1%) (Table 2). Seven MI were more susceptible by Phoenix than by BMD.

When EUCAST PK/PD BPs were applied to the MICs, none of the agents assessed met acceptable performance criteria on the Phoenix, with CA of 62.6% and 47.7% for CAZ

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and TGC, respectively (Table 3). Neither SXT nor LEV could be evaluated, as susceptible breakpoints (≤0.001 μg/ml and ≤0.5 μg/ml, respectively) were lower than the lowest reported valued on the Phoenix. CAZ testing on the Phoenix with EUCAST breakpoints was associated with 9 VME (16.7%), 8 ME (20.5%), and 23 MI (21.5%). Eight of the MI were more susceptible by the Phoenix than by BMD. TGC had 1 VME (2.0%) and 55 ME (94.8%). Only 4 isolates were categorized as susceptible by Phoenix, versus 60 by BMD.

Comparison of errors across cASTs. We determined if specific isolates were common sources of errors across multiple cAST platforms with CLSI breakpoints. For SXT, Vitek 2 yielded 25 ME that were not shared with the other platforms and 1 VME that was shared by MicroScan and Phoenix. For LVX, all platforms had 1 ME for the same isolate, and of the MI, 4 were shared by all platforms, 4 were shared by MicroScan and Phoenix, and Vitek 2 shared 3 MI with MicroScan and 4 MI with Phoenix. Additionally, Phoenix and MicroScan had 2 VME in common. For CAZ, all platforms shared 4 VME, 1 ME, and 9 MI, MicroScan and Phoenix shared 2 ME, MicroScan and Vitek 2 shared 2 MI and 4 ME, and MicroScan and Vitek 2 shared 1 ME that was an MI by Phoenix. With EUCAST PK/PD breakpoints, Vitek 2 and MicroScan shared 5 MI and 1 ME for CIP. For TGC, Vitek 2 and Phoenix shared 12 ME and 1 VME.

DISCUSSION

Most clinical laboratories in the United States rely on cASTs like Vitek 2, MicroScan Walkaway, or Phoenix to conduct AST for timely results. However, there are serious challenges when utilizing these devices for *S. maltophilia* testing. Laboratories apply CLSI BPs to data generated for *S. maltophilia* off these instruments, as FDA BPs are only available for CAZ.

In line with previous studies, MIN is the most active agent against *S. maltophilia* bloodstream isolates included in this study, with 100% of isolates categorized as susceptible by BMD (1, 3, 9). It is also the only agent that performed well on the systems evaluated against this antimicrobial, MicroScan and Phoenix, with CLSI BPs (Table 2). In contrast, no system performed acceptably for the remaining antimicrobials. Of particular concern is poor performance of SXT across all systems, demonstrating an inconsistent ability to detect resistance (MicroScan and Phoenix) or overcalling resistance in 25% of susceptible isolates (Vitek 2). This is problematic, as SXT remains the primary treatment option for infections caused by *S. maltophilia*. Twenty-five isolates were falsely categorized as resistant to SXT by the Vitek 2, of which only 4 were in EA. Recent global surveys showed that over 90% of *S. maltophilia* isolates remain susceptible to SXT (10). However, one survey of susceptibility data for *S. maltophilia* in Los Angeles County demonstrated that laboratory-reported SXT susceptibility rates ranged from 64 to 100% across institutions (11). The majority of LA County clinical laboratories in the survey used Vitek 2 for AST (J. Hindler, personal communication to R. M. Humphries), which may explain the high SXT resistance rates reported in that study (1). A prior study of the Vitek 2 did not demonstrate any VME or ME for SXT, although only 11 isolates were evaluated, 2 of which were resistant (12). A second study of *S. maltophilia* on the Vitek 2 demonstrated no VME or ME for SXT, but this study was conducted in 2001 and did not include any resistant isolates (13). Vitek 2 users should strongly consider confirming any resistant result by an alternative method prior to reporting isolates as resistant, based on the data in the present study. Conversely, the inability to detect resistant isolates is more challenging for MicroScan and Phoenix users, as a susceptible result is anticipated for *S. maltophilia*.

The Vitek 2 reported 76% of the isolates as susceptible to CAZ, which also yielded high rates of VME (44.2%) (Fig. 1). Surveillance data on *S. maltophilia* demonstrate very low susceptibility rates (<50%) for CAZ. Combined, the finding of false resistance for SXT and false susceptibility for CAZ by the Vitek 2 is a significant clinical concern, as it may drive clinicians toward use of a suboptimal antimicrobial for treatment of *S. maltophilia* infections. Overall, it must be noted that CAZ performance was poor across all systems in this study (Tables 2 and 3). This fact, combined with the low incidence of

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susceptibility to CAZ for *S. maltophilia*, limits the clinical utility of CAZ for *S. maltophilia*. At present, the CLSI recommends CAZ as an antimicrobial that warrants primary testing but may be reported only selectively, but our data, combined with those from our study of disk diffusion and gradient diffusion (which correlated equally poorly with BMD for the same collection of *S. maltophilia* isolates) (6), suggest that testing this antimicrobial is unlikely to provide clinical benefit.

LEV testing was associated primarily with MI (Table 2) by CLSI breakpoints. MI among isolates that are resistant by BMD but intermediate by a cAST may be tolerable. LEV is unlikely to be used in clinical settings to treat an isolate with an "intermediate" result, and CLSI, EUCAST, and FDA have updated LEV breakpoints for *Pseudomonas aeruginosa* which categorize an MIC of 4 μg/ml (intermediate by current CLSI *S. maltophilia* breakpoints) as resistant, leading to appropriate classification of such isolates as resistant. Similarly, PK/PD breakpoints applied by EUCAST classify these results as resistant (Table 1).

In some instances, antimicrobials without CLSI breakpoints are considered for use for infections caused by *S. maltophilia*. One option is to apply EUCAST PK/PD breakpoints to these antimicrobials. However, we found that applying PK/PD breakpoints to CAZ, LEV, CIP, and TGC MICs obtained on the cASTs evaluated in this study resulted in poor correlation with BMD results. For those agents with both CLSI and PK/PD breakpoints (CAZ, LEV), performance was worse by PK/PD interpretations. Laboratories that desire to apply EUCAST PK/PD breakpoints to drugs with no CLSI breakpoints (e.g., TGC, CIP) should be aware of these limitations, at least when using panels distributed in the United States. Alternative methods may be preferable, particularly for TGC, although gradient diffusion methods performed poorly and disk diffusion was associated with an ME of 46% in our prior study (6).

In sum, no commercial automated system performed well for *S. maltophilia*. It has long been appreciated that susceptibility testing for *S. maltophilia* is affected heavily by nuanced variation of temperature and media composition; optimization of testing conditions specific for *S. maltophilia* may be needed in order to generate results that are more comparable to those obtained by reference BMD (14–17). At present, the regulatory pathway that would enable manufacturers to obtain FDA clearance for these efforts is not existent, other than for CAZ, an antimicrobial that is often resistant. Realistically, FDA recognition of *S. maltophilia* breakpoints is unlikely in the near-term, as data required for review by FDA (e.g., contemporary PK/PD and clinical outcomes data) in order to recognize CLSI breakpoints are unavailable for this organism. Thus, addressing the rising threat of *S. maltophilia* infections requires coordination between standards development organizations, the FDA, pharmaceutical companies, and commercial diagnostic manufacturers. Laboratories should consider performing manual testing for *S. maltophilia* based on the performance of the antimicrobial being tested and resource availability (6). We previously showed that MIN, SXT, and LEV met acceptable performance criteria with disk diffusion and gradient strips, and as such, these manual methods may be preferred when testing *S. maltophilia* (6).

Limitations of this study are that BMD and commercial system testing were performed in different laboratories, although this practice is done routinely for FDA clinical trials, and that we did not test isolates systematically in replicate by all methods. We were unable to perform evaluations for all agents on all platforms, as these were not present across the Vitek 2, MicroScan, and Phoenix AST panels (e.g., MIN, CIP, and TGC). Due to growth issues, we evaluated 109 isolates on the Vitek 2, 108 isolates on the MicroScan, and 107 on the Phoenix. The majority of the isolates were collected between 2015 and 2019 at a cancer center where, as mentioned before, SXT is not used for neutropenic patients as front-line therapy due to the risk of myelosuppression. Thus, prevalence of isolates resistant to SXT is low in these patient populations and only 10 resistant isolates were included in this study. Isolates in the present study were isolated predominantly from blood cultures, whereas most *S. maltophilia* isolates are recovered from respiratory tract. In the latter scenario, *S. maltophilia* may represent

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true infection or airway colonization. Studies have shown that the populations of colonizing versus infecting *S. maltophilia* differ by strain type. It is possible that susceptibility testing results may also differ across strain types, a question worthy of future investigation (18). Nonetheless, these data provide evidence that laboratories should carefully consider the indications for testing *S. maltophilia* (i.e., focus on sterile site isolates) and also carefully review results from such testing for inconsistencies against surveillance susceptibility data (e.g., SXT resistance). The limitations of MIC results by cASTs should be discussed with local antibiotic stewardship programs and infectious diseases clinicians so that awareness of the limitations of such testing is incorporated into treatment decisions.

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