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Evaluation of the Oxoid Ceftolozane/Tazobactam (30/10) µg Antimicrobial Susceptibility Testing (AST) Disc Against the Predicate AST Disc

***A.J. Lovatt[1],** S. Bhalerao[1], E.D. Mitchell[1], D.T. Staats[2], M.M. Burgess[2], K.L. Hajek[2], N.M. Holliday[2], C.C. Knapp[2], M.R. Motyl[3].
[1]Thermo Fisher Scientific, Basingstoke, UK; [2]Thermo Fisher Scientific, Cleveland, OH, USA; [3] Merck & Co., Inc., Rahway, NJ, USA

ABSTRACT

Background
Ceftolozane/tazobactam is a dual component antibiotic developed for intravenous use in both children and adults. This combination is indicated for the treatment of Hospital-Acquired Bacterial Pneumonia, Ventilator-Associated Bacterial Pneumonia (HABP/VABP), complicated Intra-abdominal Infections (cIAI) and complicated Urinary Tract Infections (cUTI) caused by the following susceptible Gram-negative and Gram-positive microorganisms: *Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Bacteroides fragilis, Streptococcus anginosus, Streptococcus constellatus, Streptococcus salivarius, Haemophilus influenzae and Serratia marcescens.* A study was conducted to evaluate the performance and reproducibility of the new Thermo Scientific™ Oxoid™ Ceftolozane/tazobactam 30/10 µg (C/T40) Antimicrobial Susceptibility Testing (AST) disc (Thermo Fisher Scientific, Basingstoke, UK) against a predicate device disc Ceftolozane/tazobactam 30/10 µg HardyDisk™ (Hardy diagnostics, Santa Maria, CA), cleared by the Food and Drug Administration (FDA).

Methods
The Oxoid and HardyDisks were tested simultaneously against 443 clinical and challenge isolates and 17 reproducibility isolates including Enterobacterales, *Haemophilus influenzae and Pseudomonas aeruginosa.* Recommended European committee on antimicrobial susceptibility testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) quality control (QC) organisms were tested daily against 2 lots of Oxoid discs and 1 lot of HardyDisks. All isolates were tested in accordance with CLSI M02[1]/M100[2] and the EUCAST disk diffusion method[3] using FDA-cleared Mueller Hinton agar (Thermo Scientific™ Remel™ MHA) and MHA + 5% horse blood + 20 mg/L ß-Nicotinamide adenine dinucleotide (Thermo Scientific™ Oxoid™ MHF) for *Haemophilus influenzae.*

Results
Overall, a categorical agreement of 95% was achieved when the Oxoid C/T40 disc was compared to the predicate device against EUCAST breakpoints. All data showed 100% reproducibility within-reader and between-reader by calculating as the percent of results which were within 3 mm of the modal value. QC results were within the stated limits 100% of the time for each batch and reader.

Conclusions
The Oxoid C/T40 disc compared to the HardyDisk demonstrated an equivalent level of performance against EUCAST breakpoints. The high categorical agreement obtained by the Oxoid C/T40 disc indicates that this is an acceptable method for antimicrobial susceptibility testing of ceftolozane/tazobactam.

INTRODUCTION

Ceftolozane (Figure 1) is a novel cephalosporin that works simultaneously with tazobactam (Figure 2) a proven beta-lactamase inhibitor for the treatment of Hospital-Acquired Bacterial Pneumonia, Ventilator-Associated Bacterial Pneumonia (HABP/VABP), complicated Intra-abdominal Infections (cIAI) and complicated Urinary Tract Infections (cUTI).

Ceftolozane/tazobactam is indicated for intravenous treatment of adult patients (>18 years old) with HABP/VABP, caused by the following susceptible Gram-negative microorganisms: *Enterobacter cloacae, Escherichia coli, Haemophilus influenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, and Serratia marcescens.* It is also indicated for indicated the treatment of both adult and children (<18 years old) with complicated urinary tract infections (cUTI) caused by the following susceptible Gram-negative microorganisms: *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa.*

An *in vitro* study was conducted by Thermo Fisher Scientific to evaluate the performance and reproducibility of the new Ceftolozane/tazobactam (C/T40) Oxoid AST disc against a predicate device, C/T40 HardyDisk.

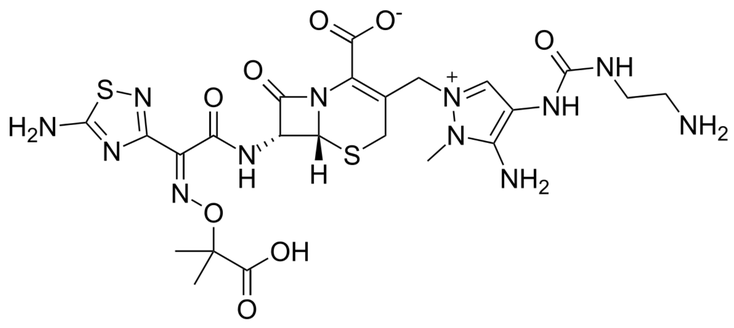

Figure 1. The chemical structure of ceftolozane.

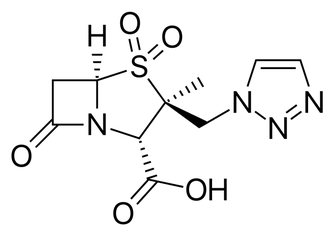

Figure 2. The chemical structure of tazobactam.

MATERIALS AND METHODS

All isolates were tested in accordance with CLSI M02[1]/M100[2] and the EUCAST disk diffusion method[3] using FDA-cleared Remel MHA and MHF for *Haemophilus influenzae*.

Colony counts were performed on inocula for at least 10% of clinical and challenge isolates and all QC and reproducibility isolates.

Quality control
Quality Control strains from the American Type Culture Collection (ATCC) were tested daily for both HardyDisks and Oxoid discs alongside clinical, challenge and reproducibility isolates to ensure all AST discs were within the QC limits (Table 1). At least 20 replicates of the quality control strains were tested per individual (3 independent individuals) to represent 3 testing sites.

The QC strains must be within specification for at least 95 % of the results during study to meet acceptance criteria[4].

Table 1. QC zone size limits for C/T40 AST discs[5].

QC organism	QC limit (mm)
Escherichia coli (ATCC® 25922)	24-32
Pseudomonas aeruginosa (ATCC® 27853)	25-31
Haemophilus influenzae (ATCC® 49766)	24-30
Escherichia coli (ATCC® 35218)	25-31
Klebsiella pneumoniae (ATCC® 700603)	17-25

Reproducibility
Two lots of Oxoid C/T40 discs were tested and read by three independent individuals against 17 indicated and on-scale reproducibility isolates over a 3-day testing period to generate a total of 306 data points.

Reproducibility must be within 3 mm of the modal zone diameter for ≥ 95 % of the results to meet acceptance criteria[4].

Clinical and Challenge isolates
One lot of Oxoid C/T40 discs was tested against one lot of C/T40 HardyDisks for a total of 443 clinical and challenge isolates including *Enterobacterales*, *Pseudomonas aeruginosa*. and *Haemophilus influenzae* (Table 2). All isolates were shared between three independent individuals (approximately 141 isolates each) to represent three testing sites which were then analysed using breakpoints set by EUCAST[6] (Table 3).

Categorical agreement (CA) must be ≥ 90 % when compared to the predicate device and the very major discrepancy and major discrepancy rate must be ≤ 3 % each to meet acceptance criteria[4].

Table 2. Number of isolates tested during the study.

Isolates	Number Tested
Clinical Isolates	305
Challenge Isolates	138
Reproducibility Isolates	17
ATCC Quality Control Strains	5
TOTAL	465

Table 3. EUCAST breakpoints for ceftolozane/tazobactam [6].

Organism(s)	Zone Diameter Interpretive Criteria (mm)		
	S ≥	R <	ATU
Enterobacterales	22	22	19-21
P. aeruginosa	23	23	-
***H. influenzae* (pneumonia)**	23	23	22-23

S= Susceptible, R= Resistant., ATU= Area of Technical Uncertainty

RESULTS

Quality Control
QC results were within the stated limits for all QC organisms (*Escherichia coli* ATCC® 25922 and ATCC® 35218, *Pseudomonas aeruginosa* ATCC® 27853, *Haemophilus influenzae* ATCC® 49766, *Klebsiella pneumoniae* ATCC® 700603) 100% of the time for each lot of Oxoid C/T40 discs.

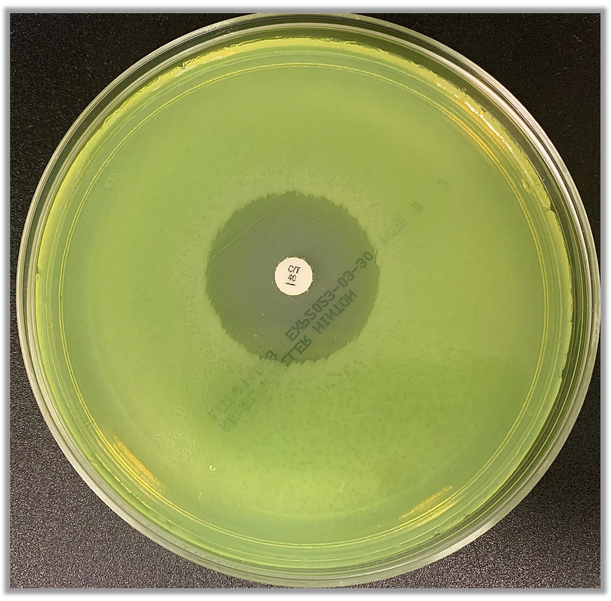

Figure 3. Oxoid C/T40 disc zone of inhibition with *Pseudomonas aeruginosa* (ATCC® 27853) on Remel MHA.

Reproducibility
The reproducibility was calculated as the percent of results which were within 3 mm of the modal value. All results showed reproducibility greater than the acceptance criteria of 95%. The summary is shown in Table 4.

Table 4. Summary of the reproducibility of Oxoid C/T40 discs between 2 lots and 3 independent individuals.

Reproducibility between disc lots			Reproducibility between individuals			
Lot 1	Lot 2	All Lots	Individual 1	Individual 2	Individual 3	All Individuals
98.7%	100%	100%	100%	99.0%	100%	99.7%

RESULTS Cont.

Clinical and Challenge isolates
The categorical agreement of the Oxoid C/T40 disc was analysed using charts such as the example shown in Figure 4 and summarised in Table 5.

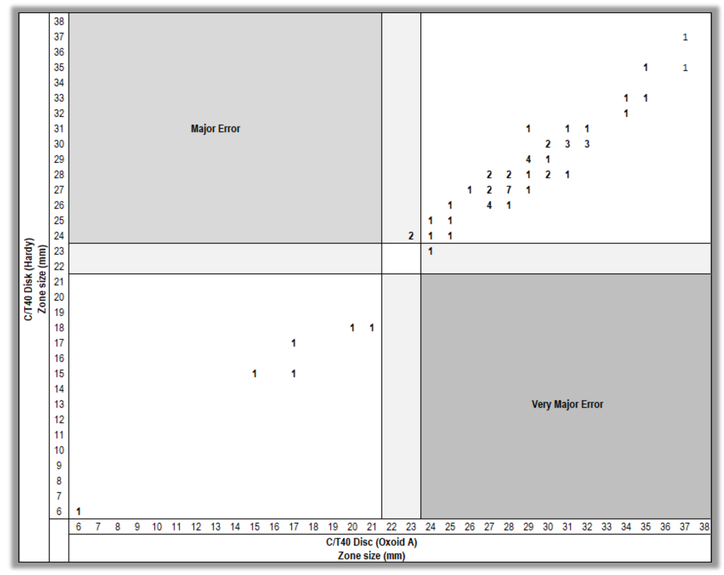

Figure 4. Analysis of the C/T40 Oxoid disc against HardyDisk for *H. influenzae* using EUCAST breakpoints.

Table 5. Analysis of C/T40 Oxoid disc vs. HardyDisk for indicated species.

	Number of isolates tested	Number of isolates in CA	% CA	Number of Minor discrepancies	% Minor discrepancies
Organism					
***Enterobacterales* combined[a]**					
Clinical	224	215	95.98 %	9	4.02 %
Challenge	100	90	90.00 %	10	10.00%
Combined	324	302	94.14 %	19	5.86 %
***Pseudomonas aeruginosa*[b]**					
Clinical	39	39	100. %	-	-
Challenge	20	20	100 %	-	-
Combined	59	59	100 %	-	-
Haemophilus influenzae					
Clinical	42	39	92.86 %	3	7.14 %
Challenge	18	18	100 %	0	0.00 %
Combined	60	57	95.00 %	3	95.00%

[a] includes *E. coli* (80), *E. cloacae* (74), *K. oxytoca* (53), *K. pneumoniae* (68) and *P. mirabilis* (49).
[b] *P. aeruginosa* does not have an ATU and therefore cannot have any minor discrepancies.

The overall categorical agreement achieved for all indicated isolates tested in this study was 95.0%, when the Oxoid C/T40 disc was compared to the predicate device.

Only 22 minor discrepancies were observed; *E. coli* (2), *E. cloacae* (12), *K. oxytoca* (3), *K. pneumoniae* (1), *P. mirabilis* (1) and *H. influenzae* (3). No major discrepancies and no very major discrepancies were observed during the study.

All colony counts were in the region of 1-2 x10^8 CFU/mL for all QC and reproducibility isolates and 10% of the clinical and challenge isolates. The average of all the colony counts combined was 1.10 x10^8 CFU/mL.

CONCLUSION

This study validates that the Oxoid C/T40 AST disc has an equivalent level of performance compared to the FDA cleared, ceftolozane/tazobactam HardyDisk in against EUCAST breakpoints. The high categorical agreement obtained by the Oxoid C/T40 disc indicates that this is an acceptable method for antimicrobial susceptibility testing of ceftolozane/tazobactam.

REFERENCES

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[4] BS EN ISO 20776-2:2007 Clinical laboratory testing and in vitro diagnostic test systems. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Evaluation of performance of antimicrobial susceptibility test devices

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[6] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 12.0, 2022

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