Eur J Clin Microbiol Infect Dis (2013) 32:621–625
DOI 10.1007/s10096-012-1785-5

ARTICLE

Comparison of Rosco Neo-Sensitabs with Oxoid paper disks in EUCAST disk diffusion antimicrobial susceptibility testing on Mueller–Hinton agar

U. S. Justesen · Z. Acar · K. Olsson · T. G. Jensen · M. B. Kerrn · R. L. Skov · B. Gahrn-Hansen

Received: 1 October 2012 / Accepted: 18 November 2012 / Published online: 12 December 2012

Abstract This study compared Neo-Sensitabs with Oxoid paper disks using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion antimicrobial susceptibility test on Mueller–Hinton agar. The EUCAST-recommended quality control strains (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212) (Part I) and clinical isolates (Part II) were investigated. In Part I of the study, 27 combinations of antimicrobial agents were tested on four quality control strains repeatedly up to 60 times and zone diameters of tablets and disks were compared. In Part II of the study, 351 clinical isolates were included to cover a broad range of species, as well as resistance mechanisms. In Part I, four major deviations (>1 mm outside quality control ranges) were observed with Neo-Sensitabs. In one case with *P. aeruginosa* ATCC 27853 (meropenem), there was a corresponding major deviation (2 mm) with the Oxoid disk. The three remaining major deviations with Neo-Sensitabs were observed with meropenem (2 mm) in *E. coli* ATCC 25922 and with ciprofloxacin (2 mm) and gentamicin (3 mm) in *P. aeruginosa* ATCC 27853. For Oxoid disks, there were only minor deviations (=1 mm outside quality control ranges) in these three cases. In Part II, there were six discrepancies, susceptible versus resistant, in 3,533 comparisons between the two methods with the clinical isolates. The Rosco Neo-Sensitabs appear to be a possible alternative to Oxoid paper disks for EUCAST disk diffusion antimicrobial susceptibility testing on Mueller–Hinton agar.

Electronic supplementary material The online version of this article (doi:10.1007/s10096-012-1785-5) contains supplementary material, which is available to authorized users.

U. S. Justesen (✉) · Z. Acar · T. G. Jensen · B. Gahrn-Hansen
Department of Clinical Microbiology,
Odense University Hospital, Winsløwparken 21, 2,
5000 Odense C, Denmark
e-mail: ujustesen@health.sdu.dk

M. B. Kerrn
SSI Diagnostica, Statens Serum Institut, Herredsvejen 2,
3400 Hillerød, Denmark

K. Olsson · R. L. Skov
Department of Microbiology and Infection Control,
Statens Serum Institut, Artillerivej 5,
2300 København S, Denmark

Introduction

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently developed a disk diffusion test for routine antimicrobial susceptibility testing [1, 2]. The method is based on two media, Mueller–Hinton agar without supplements (MH) for non-fastidious organisms, including enterococci, and MH supplemented with 5 % defibrinated horse blood and 20 mg/L β-NAD (MH-F) for *Streptococcus* spp., *Haemophilus* spp. and other fastidious organisms. Zone diameter breakpoints have been published and updated every year since December 2009, with the latest update being in January 2012 (version 2.0) [3].

The Rosco Neo-Sensitabs (Rosco Diagnostica, Taastrup, Denmark) are tablets that contain a crystalline form of the antimicrobial agent. The tablets have several advantages over paper disks, as most of the tablets can be stored for several months and used at room temperature (up to 25 °C) without degradation of the antimicrobial agent, the price is

comparable to disks and they are just as simple to work with [4]. This makes the Neo-Sensitabs a possible attractive alternative to disks. The tablets have been used for many years for antimicrobial susceptibility testing, primarily in the Scandinavian countries, in Belgium and the Netherlands [4].

The company states that the Neo-Sensitabs have been standardised according to the EUCAST minimum inhibitory concentration (MIC) breakpoints; however, very little published data exist. Recently, a study including 175 Gram-negative isolates, *Enterobacteriaceae* (n=150) and non-fermenters (n=25), demonstrated an excellent correlation between Neo-Sensitabs and Oxoid paper disks (Oxoid, Basingstoke, UK) [5]. However, no Gram-positive isolates were included. The purpose of this study was to compare Neo-Sensitabs with Oxoid paper disks using the EUCAST disk diffusion antimicrobial susceptibility test on Mueller–Hinton agar with the EUCAST-recommended strains for internal quality control (Part I) and clinical isolates (Part II), including staphylococci and enterococci.

Materials and methods

Part I

The strains recommended for internal quality control by the EUCAST for MH agar were tested, i.e. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 (Table 1). The strains were tested according to EUCAST recommendations with the antimicrobial agents listed in Table 1 [2]. A maximum of six tablets or disks were placed on each 9-cm MH agar plate. Each antimicrobial agent was tested in duplicate from one single 0.5 McFarland suspension. This was repeated on ten different days with three different batches of MH agar plates (BBL II MH agar, SSI Diagnostica, Statens Serum Institut, Hillerød, Denmark). The total number of zone diameters with each antimicrobial agent (tablet and disk) was 60. The results were compared to the EUCAST quality control targets and ranges (version 2.1 June 2012). Deviations outside the quality control range =1 mm and >1 mm were considered to be minor and major, respectively.

Part II

Routine and stored clinical isolates (n=351) were included in Part II of the study to cover a broad range of species, as well as resistance mechanisms. The 351 isolates (Table 2) were tested according to EUCAST recommendations with the same antimicrobial agents as the quality control strains, with one additional antimicrobial agent for each strain (Table 1), i.e. *Enterobacteriaceae* as *E. coli* ATCC 25922 (plus ceftriaxone 30 μg), *Pseudomonas aeruginosa* as *P. aeruginosa* ATCC 27853 (plus ceftazidime 10 μg), staphylococci as *S. aureus* ATCC 29213 (plus fusidic acid 10 μg) and enterococci as *E. faecalis* ATCC 29212 (plus vancomycin 5 μg). Extended-spectrum β-lactamase (ESBL) production, AmpC production, methicillin resistance in *S. aureus* and vancomycin resistance in enterococci were all confirmed with polymerase chain reaction (PCR), as previously described [6–8]. A tablet and disk of the same antimicrobial agent were placed on the same MH agar plate (BBL II MH agar, SSI Diagnostica). Two antimicrobial agents were placed on each plate (a total of two disks and two tablets). The EUCAST breakpoint tables version 2.0 (January 2012)

Table 1 Neo-Sensitabs and disk zone diameter median and range (n=60) with the EUCAST-recommended strains for internal quality control and the tested antimicrobial agents compared to the EUCAST quality control targets and ranges (version 2.1 June 2012)

Antimicrobial agent	*E. coli* ATCC 25922			*P. aeruginosa* ATCC 27853			*S. aureus* ATCC 29213			*E. faecalis* ATCC 29212		
	EUCAST	Rosco	Oxoid	EUCAST	Rosco	Oxoid	EUCAST	Rosco	Oxoid	EUCAST	Rosco	Oxoid
Ampicillin 2 μg										18 (15-21)	15 (14-19)	18 (16-19)
Ampicillin 10 μg	19 (16-22)	20 (18-22)	20 (18-22)									
Benzylpenicillin 1U							15 (12-18)	16 (12-17)	16 (12-17)			
Cefoxitin 30 μg	26 (23-29)	27 (23-29)	26 (23-29)				27 (24-30)	28 (25-29)	27 (25-29)			
Cefpodoxime 10 μg	26 (23-28)	26 (25-29)	26 (24-28)[a]									
Cefuroxime 30 μg	23 (20-26)	23 (21-24)	23 (21-24)									
Chloramphenicol 30 μg	24 (21-27)	23 (20-24)	23 (20-24)									
Ciprofloxacin 5 μg	35 (30-40)	34 (31-37)	34 (31-36)	29 (25-33)	29 (23-32)	29 (24-32)						
Erythromycin 15 μg							26 (23-29)	26 (25-28)[c]	26 (25-29)[c]			
Gentamicin 10 μg	23 (19-26)	23 (19-26)	23 (19-24)	20 (17-23)	23 (19-26)	22 (20-24)	22 (19-25)	24 (20-25)[c]	23 (21-24)[c]	11 (8-14)[a]	11 (9-14)	11 (10-14)
Mecillinam 10 μg	27 (24-30)	29 (26-31)	28 (25-30)									
Meropenem 10 μg	31 (28-34)	34 (29-36)	33 (28-35)	30 (27-33)	32 (28-37)	31 (27-35)						
Moxifloxacin 5 μg	32 (28-35)	31 (28-34)	31 (29-33)				28 (25-31)	29 (25-31)[c]	29 (27-30)[c]			
Nalidixic acid 30 μg	25 (22-28)	26 (24-27)	26 (25-27)									
Piperacillin-tazobactam 30+6 μg	24 (21-27)	25 (23-26)	25 (22-26)	26 (23-29)	28 (26-30)	27 (26-29)						
Tigecycline 15 μg							22 (19-25)	21 (19-23)[c]	21 (19-24)[c]	23 (20-26)	21 (20-23)	22 (20-23)
Trimethoprim 5 μg	25 (21-28)	25 (23-27)[b]	26 (23-27)[b]				25 (22-28)	24 (21-25)[b]	25 (23-26)[b]			

*Version 1.1 September 2009. a: n=59. b: n=56. c: n=58. In grey and marked with bold are the deviations from the EUCAST quality control range

Table 2 Species and number of clinical isolates (n=351)

Enterobacteriaceae (170)	*Pseudomonas aeruginosa* (30)
Citrobacter freundii (10)	Staphylococci (100)
Citrobacter koseri (10)	MSSA (40)
E. coli (70)	MRSA (10)
- Non-ESBL and non-AmpC (50)	*Staphylococcus capitis* (1)
- ESBL and/or AmpC (20)	*Staphylococcus epidermidis* (33)
Enterobacter cloacae (10)	*Staphylococcus haemolyticus* (1)
Klebsiella oxytoca (10)	*Staphylococcus hominis* (5)
Klebsiella pneumoniae (30)	*Staphylococcus lugdunensis* (3)
- Non-ESBL (20)	*Staphylococcus saprophyticus* (7)
- ESBL (10)	Enterococci[a] (51)
Proteus mirabilis (10)	*Enterococcus casseliflavus* (1)
Proteus vulgaris (10)	*Enterococcus faecalis* (21)
Serratia liquefaciens (2)	*Enterococcus faecium* (27)
Serratia marcescens (8)	*Enterococcus gallinarum* (2)

ESBL: extended-spectrum β-lactamase; MSSA: methicillin-susceptible *S. aureus*; MRSA: methicillin-resistant *S. aureus*

[a] Vancomycin-resistant enterococci: *Enterococcus casseliflavus* (1), *Enterococcus faecalis* (1), *Enterococcus faecium* (7), *Enterococcus gallinarum* (2)

were used, except for the following combinations: enterococci and ampicillin 2 μg: $S \geq 10$ mm and $R=9$ mm, and enterococci and gentamicin 10 μg: $S \geq 10$ mm and $R=9$ mm (the Neo-Sensitabs gentamicin 30 μg were not available at the time of this study). We defined these breakpoints as the Neo-Sensitabs have a diameter of 9 mm. Therefore zones around the Oxoid disks with diameters below 9 mm were read as 9 mm in order to be able to compare zone diameters. Zone diameters were measured using an electronic calliper. If isolates were categorised differently, susceptible or resistant (SR discrepancy), with the two methods according to the EUCAST breakpoint tables for the interpretation of zone diameters, the discrepancy was resolved with MIC testing (Etest, bioMérieux, Craponne, France) and/or PCR, as previously described. Scatterplots of the measured zone diameters and linear regression lines were constructed using Stata 9.2 (Stata Corp LP, College Station, TX, USA).

Results

Part I

The results are presented as medians and ranges in Table 1. In ten cases, the Neo-Sensitabs range deviated outside the EUCAST range [9], but in six of those cases, it was =1 mm (minor deviation). The Oxoid disk range deviated from the EUCAST range in five cases, of which four were =1 mm. In all the cases with Oxoid disk deviations, there were also similar deviations with the Neo-Sensitabs. Four major deviations (>1 mm) were observed with the Neo-Sensitabs. In one case with *P. aeruginosa* ATCC 27853, there was a corresponding major deviation with the Oxoid disk. The Neo-Sensitab and Oxoid disk deviation were 4 mm and 2 mm, respectively, for meropenem (Table 1). The three remaining major deviations with the Neo-Sensitabs were observed with meropenem (2 mm) in *E. coli* ATCC 25922 and with ciprofloxacin (2 mm) and gentamicin (3 mm) in *P. aeruginosa* ATCC 27853. For Oxoid disks, there were only minor deviations in these three cases (Table 1).

Part II

The total number of comparisons was 3,533. One comparison with trimethoprim on an *S. aureus* isolate was not performed (the Oxoid disk did not attach to the agar surface and dropped off unnoticed when the agar was turned upside down). Scatterplots of the measured zone diameters with a linear regression line and an identity line (X=Y) for each bacterial group and antimicrobial agent are available in the Supplementary material. There were six SR discrepancies between the two methods (Table 3). One *Klebsiella pneumoniae* isolate was categorised S towards cefoxitin with the Neo-Sensitab and as R with the Oxoid disk (possible AmpC producer). However, the isolate was AmpC negative (and was categorised as S to cefuroxime). Two SR discrepancies were observed with cefpodoxime and cefuroxime in two *Citrobacter freundii* isolates. MIC testing was not performed as the cefpodoxime EUCAST MIC breakpoint is for uncomplicated urinary tract infections only and cefuroxime does not have a EUCAST MIC breakpoint for *C. freundii*. Furthermore, according to the EUCAST Expert rules, testing with and the use of third-generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime is controversial in *Citrobacter freundii* [10]. There were 23 IS or IR (intermediate versus susceptible or resistant) discrepancies. Sixteen of these discrepancies were with piperacillin–tazobactam and *Enterobacteriaceae* and, in all 16 cases, the Neo-Sensitab zone was larger than the Oxoid disk zone (1–4 mm). The isolates were *E. coli* (9), *K. pneumoniae* (5), of which 12 were ESBL positive, and one isolate of *Enterobacter cloacae* and *K. oxytoca*. MIC testing was performed in 13 cases of the piperacillin–tazobactam discrepancies and categorisation was correct in five cases with Neo-Sensitabs and in eight cases with Oxoid disks. In the remaining seven IS or IR discrepancies, not involving piperacillin–tazobactam, MIC testing was performed in five cases, and categorisation was correct in four cases with Neo-Sensitabs and in one case with Oxoid disks. A single isolate of *Enterococcus gallinarum* was categorised as vancomycin susceptible by both the Neo-

Table 3 SR discrepancies between the Neo-Sensitabs and Oxoid disks

Species	Tablet/disk	Rosco	Oxoid	Comment
Klebsiella pneumoniae	Cefoxitin 30 μg	S	R	AmpC negative → S
Proteus mirabilis	Ampicillin 10 μg	S	R	MIC: 12 mg/L → R
Staphylococcus epidermidis	Cefoxitin 30 μg	S	R	MecA positive → R
Staphylococcus aureus	Fusidic acid 10 μg	R	S	23 mm versus 24 mm[a]
Citrobacter freundii	Cefpodoxime 10 μg	S	R	21 mm versus 20 mm[b]
Citrobacter freundii	Cefuroxime 30 μg	R	S	17 mm versus 18 mm[b]

[a] The breakpoint was changed from 22/22 to 24/24 mm after the study had finished. As the study was performed with the old breakpoint, no discrepancy was initially present and MIC testing was not performed

[b] MIC testing was not performed as there is no EUCAST MIC breakpoint

Sensitab and the Oxoid paper disk. However, the isolate had previously been categorised as vancomycin resistant by MIC testing with an MIC of 8 mg/L.

Discussion

The results from Part I of our study with 27 comparisons demonstrated six minor deviations only, i.e. =1 mm from the EUCAST ranges, of which five were with the Neo-Sensitabs. Although this could indicate that these specific tablets need some adjustment, the only apparent systematic error was the ampicillin 2-μg tablet. The median zone diameter with *E. faecalis* ATCC 29212 and the ampicillin 2-μg tablet was 15 mm, which is the same as the lower end of the EUCAST quality control range (15–21 mm), indicating that half of the zone diameters were below the range. After the study had finished, we were informed by Rosco Diagnostica that the batch used for the study had been recalled, because of too low concentrations of ampicillin in the tablets, which could explain the observed errors. None of the minor deviations resulted in SR discrepancies in Part II of the study. The four major deviations (>1 mm) with the Neo-Sensitabs were matched by similar Oxoid disk deviations. Inhibition zones for aminoglycosides above or below the EUCAST range with *P. aeruginosa* ATCC 27853 have been described before and are probably explained by too high or too low concentrations of divalent cations in the MH agar [2]. The meropenem and ciprofloxacin inhibition zones above and below the EUCAST range are more difficult to explain. However, double zones are often seen when testing meropenem and ciprofloxacin, with fine growth that may appear as an inner zone [2]. The EUCAST recommendation is that "Zone edges should be read at the point of complete inhibition as judged by the naked eye." [2]. It is possible that an inner zone was present and not included in the meropenem zone diameter but incorrectly included in the ciprofloxacin zone diameter. This would result in zone diameters above and below the range. All in all, 6.7 % of the Neo-Sensitabs zone diameters and 3.2 % of the Oxoid disk zone diameters were outside the range. Part II of the study demonstrated that the Neo-Sensitabs also worked very well with clinical isolates and resulted in SIR categorisation in accordance with the Oxoid paper disk, with very few exceptions.

There are some caveats in our study. We did not include internal quality control strains for the detection of specific resistance mechanisms. The EUCAST-recommended strains for this purpose with quality control targets and ranges are being tested at the moment, e.g. *E. coli* ATCC 35218 (TEM-1 β-lactamase producer) and *S. aureus* NCTC 12493 (oxacillin hetero-resistant, *mecA* positive) but were not available for this study. However, we included clinical isolates with specific resistance mechanisms such as ESBLs, AmpC, methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci, and they were all detected, apart from a single *Enterococcus gallinarum* isolate. Vancomycin resistance can be difficult to detect using disk diffusion because the laboratory technicians have to differentiate between sharp and fuzzy vancomycin zone edges. Finally, we did not perform comparisons on the MH-F agar or on other types of MH agar, and the results and conclusions from this study are only applicable to the BBL II MH agar.

In summary, this study demonstrated six minor deviations (=1 mm) with the Neo-Sensitabs in zone diameter ranges for the internal quality control strains. There were four major deviations (>1 mm) and, in these four cases, there were also similar deviations with the Oxoid disks. An apparent systematic error with the ampicillin 2-μg tablet was observed, resulting in zone diameters below the range. There were six SR discrepancies between the Neo-Sensitabs and Oxoid disks with the clinical isolates. The Rosco Neo-Sensitabs appear to be a possible alternative to Oxoid paper disks for EUCAST disk diffusion antimicrobial susceptibility testing on Mueller–Hinton agar.

Conflict of interest The Department of Clinical Microbiology at Odense University Hospital (U. S. Justesen, Z. Acar, T. G. Jensen, B. Gahrn-Hansen) received research funding from Rosco Diagnostica for Part II of the study. R. L. Skov has received funds for presenting data from Part I of the study from Rosco Diagnostica. Rosco Diagnostica did not participate in the study design, in the collection, analysis or interpretation of the data, or in the writing of this paper.

References

1. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2012) EUCAST web site. http://www.eucast.org. Accessed 21 Sept 2012
2. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2012) EUCAST Disk Diffusion Test Methodology. http://www.eucast.org/antimicrobial_susceptibility_testing/disk_diffusion_methodology/. Accessed 21 Sept 2012
3. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2012) Clinical breakpoints. http://www.eucast.org/clinical_breakpoints. Accessed 21 Sept 2012
4. Lauwers S, Philippe J, Van Zeebroeck A, Pierard D, Derde MP, Kaufman L (1991) Quality control in antimicrobial disk susceptibility testing: a Belgian multicenter study. Eur J Clin Microbiol Infect Dis 10(8):652–656
5. Rodríguez-Villalobos H, Boeras A (2012) Comparison of Neosensitabs (ROSCO) tablets with paper discs (OXOID) for antimicrobial susceptibility testing of Gram-negative clinical isolates according to the EUCAST recommendations. Clin Microbiol Infect 18(Suppl 3):S119
6. Hansen DS, Schumacher H, Hansen F, Stegger M, Hertz FB, Schønning K, Justesen US, Frimodt-Møller N; DANRES Study Group (2012) Extended-spectrum β-lactamase (ESBL) in Danish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*: prevalence, β-lactamase distribution, phylogroups, and co-resistance. Scand J Infect Dis 44(3):174–181
7. Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR (2012) Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecA*(LGA251). Clin Microbiol Infect 18(4):395–400
8. Lester CH, Olsen SS, Schønheyder HC, Hansen DS, Tvede M, Holm A, Arpi M, Friis-Møller A, Jensen KT, Kemp M, Hammerum AM (2010) Typing of vancomycin-resistant enterococci obtained from patients at Danish hospitals and detection of a genomic island specific to CC17 *Enterococcus faecium*. Int J Antimicrob Agents 35(3):312–314
9. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2012) EUCAST Quality Control. http://www.eucast.org/antimicrobial_susceptibility_testing/qc_tables/. Accessed 21 Sept 2012
10. Leclercq R, Cantón R, Brown DF, Giske CG, Heisig P, Macgowan AP, Mouton JW, Nordmann P, Rodloff AC, Rossolini GM, Soussy CJ, Steinbakk M, Winstanley TG, Kahlmeter G (2011) EUCAST expert rules in antimicrobial susceptibility testing. Clin Microbiol Infect [Epub ahead of print]. doi:10.1111/j.1469-0691.2011.03703.x