Veterinary Microbiology 214 (2018) 56–64

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Relative performance of antimicrobial susceptibility assays on clinical *Escherichia coli* isolates from animals

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ARTICLE INFO

Keywords:
Disc diffusion
Broth-microdilution
Accuracy
ROC
Antimicrobial resistance
Surveillance

ABSTRACT

The assessment of antimicrobial resistance in bacteria derived from animals is often performed using the disc diffusion assay. However broth-microdilution is the preferred assay for national antimicrobial resistance surveillance programs. This study aimed to evaluate the accuracy of disc diffusion relative to broth-microdilution across a panel of 12 antimicrobials using data from a collection of 994 clinical *Escherichia coli* isolates from animals. Disc diffusion performance was evaluated by diagnostic sensitivity, specificity, likelihood ratio pairs and receive-operating characteristic (ROC) analysis. Data was dichotomised using CLSI susceptible and resistant clinical breakpoints. In addition, disc diffusion breakpoints produced using diffusion Breakpoint Estimation Testing Software (dBETS) were evaluated. Analysis revealed considerable variability in performance estimates for disc diffusion susceptible and resistant breakpoints (AUC ranges: 0.78–0.99 and 0.92–1.0, respectively) across the panel of antimicrobials. Ciprofloxacin, tetracycline, and ampicillin estimates were robust across both breakpoints, whereas estimates for several antimicrobials including amoxicillin-clavulanic acid, cefoxitin and gentamicin were less favourable using susceptible breakpoints. Overall performance estimates were moderately improved when dBETS susceptible breakpoints were applied. For most antimicrobials, disc diffusion was accurate at predicting resistance of clinical *E. coli* from animals that could otherwise be determined by broth-microdilution. While disc diffusion is suboptimal for assessing the proportion of fully susceptible isolates for some drugs, sensitivity and specificity estimates provided here allow for the use of standard formula to correct this. For this reason, disc diffusion has applicability in national surveillance provided the performance of the assay is taken into account.

1. Introduction

The emergence and spread of bacteria resistant to multiple antimicrobials including 'last-line of defence' drugs is a critical threat to the well-being of humans, animals and the environment. Strong international consensus for global action on antimicrobial resistance (AMR) has been established within the United Nations General Assembly (United Nations, 2016) and international agencies responsible for human health, animal health and agriculture (OIE, 2015; WHO, 2015b). National surveillance programs are the cornerstone in global efforts to contain the spread of AMR (WHO, 2015a). Integrated national surveillance involving the coordinated collection of data on AMR in humans, animals and the environment is critical for detecting emerging forms of resistance and evaluating the success of policies designed to contain AMR (Laxminarayan et al., 2013).

Surveillance of AMR in animal-derived bacteria is typically focussed on commensal and zoonotic bacteria from food-producing animals rather than clinical isolates from diseased animals. While zoonotic

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https://doi.org/10.1016/j.vetmic.2017.12.008
Received 8 June 2017; Accepted 11 December 2017

Table 1
Disc diffusion and broth-microdilution interpretative criteria for twelve antimicrobials evaluated in this study and applied to 994 clinical *Escherichia coli* isolates derived from animals.

		Susceptible Breakpoints		Resistant Breakpoints		
Antimicrobial	Abbreviation	Disc diffusion zone diameter (mm)	Broth-microdilution MIC (μg/ml)	Disc diffusion zone diameter (mm)	Broth-microdilution MIC (μg/ml)	MIC range (μg/ml)
Amoxicillin-clavulanic acid	AMC	≥18[a]	≤8[a]	≤13[a]	≥32[a]	1.0–64
Amikacin	AMK	≥17[a]	≤16[a]	≤14[a]	≥64[a]	0.5–64
Ampicillin	AMP	≥17[a]	≤8[a]	≤13[a]	≥32[a]	1.0–128
Cephalothin	CEF	≥18[a]	≤8[a]	≤14[a]	≥32[a]	2.0–128
Ceftiofur	CFT	≥21[a]	≤2[a]	≤17[a]	≥8[a]	0.06–64
Ciprofloxacin	CIP	≥21[b]	≤1[b]	≤15[b]	≥4[b]	0.008–8
Cefovecin	CVN	≥23[c]	≤2[c]	≤19[c]	≥8[c]	0.12–128
Cefoxitin	FOX	≥18[b]	≤8[b]	≤14[b]	≥32[b]	1.0–128
Gentamicin	GEN	≥16[a]	≤2[a]	≤12[a]	≥8[a]	0.12–64
Imipenem	IPM	≥23[a]	≤1[a]	≤19[a]	≥4[a]	0.06–4
Trimethoprim-sulfamethoxazole	SXT	≥16[a]	≤2[a]	≤10[a]	≥4[a]	0.12–16
Tetracycline	TET	≥19[a]	≤4[a]	≤14[a]	≥16[a]	0.12–128

[a] Derived from CLSI VET01-S3.
[b] Derived from CLSI M100-S25.
[c] Cefovecin breakpoints based on manufacturer's recommendation.

Table 2
Diagnostic performance estimates of disc diffusion relative to broth-microdilution for 994 clinical *Escherichia coli* isolates from animals using CLSI susceptible and resistant breakpoints. DSe, diagnostic sensitivity; DSp diagnostic specificity; AUC, area under the curve. Exact 95% confidence intervals are given in Supplementary materials.

	Susceptible Breakpoint Estimates			Resistant Breakpoint Estimates		
Antimicrobial	Relative DSe	Relative DSp	AUC	Relative DSe	Relative DSp	AUC
Amoxicillin-clavulanic acid	0.23	0.99	0.82	0.79	0.99	0.98
Amikacin	NA	0.99	NA	NA	1.0	NA
Ampicillin	0.93	0.81	0.96	0.97	0.95	0.98
Cephalothin	0.70	0.81	0.82	0.75	0.98	0.92
Ceftiofur	0.84	0.99	0.94	0.94	0.99	0.98
Ciprofloxacin	0.96	1.0	0.99	0.99	1.0	1.0
Cefovecin	0.67	0.96	0.87	0.88	0.99	0.97
Cefoxitin	0.33	1.0	0.78	0.83	0.99	0.97
Gentamicin	0.50	0.99	0.82	0.92	1.0	0.97
Imipenem	NA	0.99	NA	NA	1.0	NA
Trimethoprim-sulfamethoxazole	0.70	0.99	0.93	0.72	0.99	0.94
Tetracycline	0.93	0.98	0.97	0.95	0.99	0.98

NA, not available due to insufficient data for the analysis.

bacteria such as *Salmonella* spp. and *Campylobacter* spp. pose the greatest health threat to humans, commensal organisms of the gastrointestinal tract such as *Escherichia coli* are also considered high-risk for the transmission of antimicrobial resistance genes to human bacteria via food products (Shaban et al., 2014). A barrier to achieving comprehensive surveillance of all AMR risks in animals is the acquisition of data from a sufficient number of clinical isolates. This could be overcome by collecting antimicrobial assay results from veterinary laboratories either as minimum inhibitory concentration (MIC) from dilution-based assays or millimetres of zone diameter from diffusion-based assays. The MIC is widely considered to be the superior measure for quantifying an isolate's susceptibility to antimicrobials (Turnidge and Paterson, 2007), and hence, broth-microdilution is the preferred susceptibility assay for national surveillance programs (ISO, 2006; OIE, 2017b). However, disc diffusion is often favoured by veterinary laboratories as it is affordable and readily customisable for a range of animal pathogens. There is considerable scope to merge susceptibility data acquired from disc diffusion from multiple laboratories into national surveillance provided the results are comparable to those from MIC assays.

The overall accuracy of disc diffusion relative to broth-microdilution remains inconclusive despite several previous studies having evaluated the assay's performance across a range of bacterial species and antimicrobials (Benedict et al., 2013; Hoelzer et al., 2011; Klement et al., 2005; Rhodes et al., 2014; Saini et al., 2011; Schumacher et al., 2001). This may be due to limitations of isolates entering such studies including small sample size, study validity (i.e. isolates are not obtained from an epidemiologically relevant population from which inferences can be drawn) and low prevalence of resistance to antimicrobials, particularly those that are critically important to humans. For instance, of those studies which include animal-derived *E. coli*, only Benedict et al. (2013) (n = 3362), Klement et al. (2005) (n = 231) and Rhodes et al. (2014) (n = 304) assessed more than 200 isolates. Many previous studies have also constrained the evaluation of test performance to descriptive measures such as observed agreement of dichotomous results, simple linear regression and error-rate bounding without considering modern statistical approaches that fully exploit the data to aid interpretation of test performance.

Inevitably the assessment of diagnostic test accuracy relies on the reference test (usually broth-microdilution) and the cut-point (or breakpoint) used to dichotomise the data. In the context of AMR, the *clinical* breakpoint may define full susceptibility (susceptible breakpoint), resistance (resistant breakpoint) or the non-susceptible population (i.e. the combination of resistant and intermediate isolates) based on available pharmacokinetic data. In the evaluation of disc diffusion performance, some studies have applied the resistant breakpoint (Benedict et al., 2013; Hoelzer et al., 2011) while others applied the susceptible breakpoint (Klement et al., 2005; Saini et al., 2011). Inevitably different breakpoints will yield different estimates of test accuracy, with a resultant trade-off between the two types of