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How difficult is it to achieve compliance with the quality control requirements of the Clinical and Laboratory Standards Institute's guideline M42-A?

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Abstract

Data accumulated in a multi-laboratory study of disc diffusion susceptibility data for *Escherichia coli* ATCC 25922 was analysed with respect to the acceptable ranges for this strain specified in the Clinical and Laboratory Standards Institute's M42-A guidelines. Seven laboratories each generated 60 zone size measurements for discs containing five agents for which acceptable ranges had been published. Of the 35 data sets analysed 37% contained >10% of their measurements outside the acceptable ranges. For each agent, between one and four of the seven data sets obtained contained >10% of the zone sizes outside the acceptable ranges. Only one laboratory obtained data sets that for all agents, contained <10% of the zone sizes outside the acceptable. For the other six, non-compliant data sets were obtained for between one and four of the agents. These data suggest that the adherence to a specified test protocol cannot be assumed to result in the achievement of compliance with the quality control requirements of that protocol. They would further suggest that the need to undertake corrective actions, as specified in M42-A, would be frequently required before a laboratory could claim to be using this protocol and could legitimately apply any cut-off values or breakpoints that are or that might be, associated with it use. As laboratories involved in susceptibility testing of bacteria associated with aquaculture frequently handle only a small number of strains per year, the achievement of compliance with the quality control requirements of a standard protocol might require a disproportionate and unacceptable increase in their work load. The data obtained in this retrospective study also demonstrated that, even if all data sets containing >10% non-compliant measurements were eliminated; significant inter-laboratory variation in mean zone sizes would remain.

Laboratory-independent cut-off values will be of value only if inter-laboratory variation in disc diffusion data can be reduced to a minimum. It is argued that the data presented here provides strong grounds for questioning whether, particularly in the case of laboratories with small strain throughputs, inter-laboratory variation can be appropriately resolved by rigorous specifications of test protocols and quality control criteria. Thus, they would rather support the development of cut-off values by the application of normalised resistance interpretation. As the cut-off values generated by this approach are laboratory-specific, they are not influenced by inter-laboratory variation.

Keywords: Disc diffusion; Epidemiological cut-off values; M42-A; Normalised resistance interpretation; Inter-laboratory variation

1. Introduction

A central problem associated with setting interpretive criteria for the data generated by disc diffusion antimicrobial susceptibility tests is the degree of variation, particularly inter-laboratory variation, that is encountered in the data they generate (Kronvall et al., 1988, 2003). NicGabhainn et al. (2003), Huys et al. (2005) and Smith et al. (2007) have all demonstrated that inter-laboratory variation is also a problem in studies of bacteria associated with aquaculture. There are essentially two approaches to this problem.

One approach involves the attempt to reduce the extent of inter-laboratory variation by rigorous standardisation of the test protocol and the provision of compliance criteria via standard quality control (QC) procedures that include acceptable ranges for control strains. The M42-A protocols recently published by the Clinical and Laboratory Standards Institute (CLSI, 2006a) are currently the most developed standard protocols for disc diffusion susceptibility testing of bacteria associated with aquatic animals. The assumption underlying this approach is that,

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Table 1
Percentage of zone size determinations that lay above or below the acceptable range specified in M42-A (CLSI, 2006a), analysed by agent and laboratory

LAB	AMP[a]		SFT		FLO		OTC		OXA	
	Below	Above	Below	Above	Below	Above	Below	Above	Below	Above
1	0	0	17	0	0	0	0	0	20	0
2	0	2	0	7	0	0	0	53	0	0
3	0	0	0	0	0	0	0	0	27	0
4	0	0	0	0	0	0	0	5	9	0
5	0	22	0	8	0	2	0	42	0	0
6	0	0	38	0	12	0	27	2	33	0
7	0	61	2	0	0	12	0	0	10	0

All 35 data sets contained 60 zone size measurements.
[a] Abbreviations as in Methods section.

having rigorously specified the test protocol and its associated QC procedures, a set of laboratory-independent, 'universal' breakpoints or epidemiological cut-off values can be established that could validly be applied to data generated in any laboratory that employs that protocol (Miller and Reimschuessel, 2006).

A second approach is based on the assumption that inter-laboratory variation can be reduced but not eliminated by the application of standard protocols (Kronvall et al., 1988). In this approach, the goal of 'universal', laboratory-independent cut-off values is abandoned in favour of adopting a standard method of establishing laboratory-specific values. Normalised resistance interpretation (NRI) represents the most sophisticated method that has been developed (Kronvall, 2003; Kronvall et al., 2003; Joneberg et al., 2003) for estimating laboratory-specific epidemiological cut-off values from a consideration of disc diffusion data without reference to those generated by minimum inhibitory concentration (MIC) tests.

In aquaculture, the debate as to whether laboratory-independent or laboratory-specific breakpoints will be most effective in reducing errors in the interpretation of disc diffusion data has only recently been started (Smith et al., 2007). It is probable that it will only be resolved by continuing to examine the consequences that arise from each approach. Two issues relevant to this debate will be the ease with which laboratories can achieve compliance with the acceptable rages for control strains specified in M42-A (CLSI, 2006a) and the extent of the residual inter-laboratory variation that will remain after such compliance has been achieved.

This paper presents information relevant to these two issues that was generated by a re-analysis of the data originally presented by NicGabhainn et al. (2003). These authors reported on the zone sizes recorded by seven laboratories during the susceptibility testing of the control strain *E. coli* ATCC 25922 using the disc diffusion protocols of Alderman and Smith (2001). It should be noted that the study reported by NicGabhainn et al. (2003) was performed before the acceptable ranges associated with M42-A (CLSI, 2006a) had been published. However, with respect to Group 1 organisms, such as *E. coli* that can grow on Mueller–Hinton agar at 22 C within 48 h, these protocols are functionally identical to those specified in M42-A (CLSI, 2006a). It is therefore legitimate to apply the acceptable ranges for *E. coli* ATCC 25922 published in M42-A (CLSI, 2006a) to data obtained using the Alderman and Smith (2001) protocols.

2. Methods

Full details of the methods used to generate the data analysed here are provided in NicGabhainn et al. (2003), who, with respect to the design of their study, followed the recommendation of National Committee on Clinical Laboratory Standards (NCCLS, 1999). As participation in this study was governed by a confidentiality agreement, the seven laboratories that took part in this work have been identified here only by a randomly assigned number. All of the seven participating laboratories measured the zone sizes generated by the use of discs containing 10 μg ampicillin (AMP), 25 μg amoxycillin (AMX), 30 μg florfenicol (FLO), 30 μg flumequine (FLU), 30 μg oxytetracycline (OTC), 2 μg oxolinic acid (OXA) and 25 μg trimethoprim/sulfmethoxazole (1:19) (SFT) against *E. coli* ATCC 25922. The disc diffusion test protocol employed was that of Alderman and Smith (2001) which, for this organism, is functionally identical with that specified in M42-A (CLSI, 2006a). Incubations were at 22±2 °C and zones were read after 44–48 h. With respect to flumequine, Laboratory 4 performed 40 measurements. With this exception, all the other 48 data sets analysed comprised of 60 independent measurements.

3. Results

M42-A (CLSI, 2006a) provides acceptable ranges for five of the seven discs used in NicGabhainn et al. (2003). Table 1 presents, for each of the seven laboratories, the percentage of the 60 zone size measurements they made using these five discs that were outside the acceptable ranges. Of the 35 data sets analysed in Table 1, 15 (43%) lay completely within the acceptable range, 4 (11%) contained between

Table 2
Minimum and maximum values and the spread of the mean values (mm) established by the seven laboratories for the zones generated by seven discs against *E. coli* ATCC 25922

Agent[a]	All labs				Labs with <10% zone sizes outside acceptable range			
	n	Mean (mm)			*n*	Mean (mm)		
		Min	Max	Spread		Min	Max	Spread
AMP	7	16	23	8	5	16	20	5
AMX	7	19	25	7	na	na	na	na
FLO	7	23	31	9	5	24	29	6
FLU	7	31	47	17	na	na	na	na
OTC	7	27	36	10	4	28	31	4
OXA	7	28	36	9	3	30	36	7
SFT	7	26	34	9	5	30	34	5

na; as no acceptable ranges have been published for these discs these calculations are not applicable.
[a] Abbreviations as in Methods section.