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Breakpoints for disc diffusion susceptibility testing of bacteria associated with fish diseases: A review of current practice

Pete Smith

Fish Disease Group, Department of Microbiology, National University of Ireland, Galway, Ireland

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Abstract

A survey of the methods being employed to determine antimicrobial susceptibility of bacteria associated with aquaculture was performed on behalf of the Permanent Advisory Network for Diseases in Aquaculture. Thirty-two laboratories in 18 countries responded and 25 reported the breakpoints they used for disc diffusion assays applied to Group 1, non-fastidious organisms isolated from finfish. A total of 117 breakpoints were reported for assays in which the disc contents were those specified by the current standard protocols. Data on the source of these breakpoints and the confidence the laboratories had in them are presented. Overall there was a considerable variation in the breakpoints employed by different laboratories and this variation is discussed in terms of the inter-laboratory precision that can be expected from the application of disc diffusion protocols. This paper discusses the possible clinical significance of the variations in the breakpoints and, where there are available data, the extent to which those in use are consistent with breakpoints suggested by other approaches.

The data presented in this paper represent a starting point for the movement towards harmonising breakpoints used in association with the standard disc diffusion protocols that have been proposed for susceptibility testing of bacteria associated with fish diseases.

Keywords: Antimicrobial susceptibility; Disc diffusion; Breakpoints; Finfish; PANDA

1. Introduction

Antimicrobial susceptibility testing of bacteria has two components. The first is the generation of laboratory data by the application of a specific test protocol and the second is the interpretation of those data by the application of breakpoints. A survey of methods, protocols and breakpoints currently in use to investigate bacteria associated with fish disease has recently been carried out for the Permanent Advisory Network for Diseases in Aquaculture (PANDA). Smith (in press) has presented an analysis of the methods and protocols being used by the 32 laboratories that responded to this survey. This demonstrated that the large majority (91%) of responding laboratories used disc diffusion methods to investigate the susceptibility of clinical isolates.

A set of closely related disc diffusion test protocols for aquatic bacteria has been published recently. Those of Alderman and Smith (2001) were developed from the NCCLS M31-T (NCCLS, 1997) protocols for veterinary bacteria and the Clinical and Laboratory Standards Institute (CLSI) M42-P protocols (CLSI, 2005) were themselves developed from Alderman and Smith (2001). Seventy five percent of the laboratories responding to the

E-mail address: peterrrsmith@eircom.net.

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Table 1
Species-dependent breakpoints (mm) used to determine resistance in one laboratory

Agent	*A. salmonicida*	*Y. ruckeri*	*Vibrio* sp.
Amoxycillin	16	20	14
Oxytetracycline	34	18	20
Trimethoprim/ sulfamethoxazole	8	24	24

PANDA survey used one of this family of closely related protocols (Smith, in press). This demonstrates that there is a significant degree of harmony in the protocols being used for disc diffusion susceptibility testing.

Both M42-P (CLSI, 2005) and Alderman and Smith (2001) classified organisms that could grow within 48 h on unmodified Mueller–Hinton agar at 22±2 °C or 28±2 °C in Group 1. This paper presents an analysis of and commentary on the breakpoints being used by the laboratories that responded to the PANDA survey when they were testing the susceptibility of Gram-negative, Group 1 organisms isolated from finfish.

2. Methods

The data analysed in this paper were obtained in an e-mail survey of laboratories. The questionnaire used in this survey can be consulted on the PANDA web site (http://www.europanda.net). Smith (in press) has reported details of the laboratories that responded and the methods and protocols that they employed to perform susceptibility tests.

3. Results

3.1. General characteristics of the data

A total of 32 laboratories from 18 countries responded to the survey. Three reported the use of MIC methods only. Of the 29 laboratories that reported using disc diffusion methods, 2 reported that they had insufficient data to set breakpoints and a further 2 laboratories serviced shrimp farms. This paper will be confined to an analysis of the breakpoint data collected from the 25 laboratories that reported using disc diffusion protocols to assess the susceptibility of Gram-negative, Group 1 organisms (CLSI, 2005) isolated from finfish.

Of the 25 laboratories, 11 used the protocols of Alderman and Smith (2001), 4 used M42-P (CLSI, 2005) and 3 used modifications of M31-A2 (CLSI, 2003). Of the other 7 laboratories, only 1 used an agar other than Mueller–Hinton agar. The number of antimicrobial agents to which susceptibility was investigated varied between laboratories over a range of 2–11 with a median of 6.

Appendix 1 of M42-P (CLSI, 2005) provides a list of the recommended disc content for 18 antimicrobial agents. In addition, Alderman and Smith (2001) recommended the use of flumequine discs containing 30 μg. Of the 157 breakpoints that were reported for Gram negative, Group 1 organisms by the 25 laboratories servicing finfish farms, 117 were for discs with the contents recommended in these protocols. Of the other breakpoints, 9 were associated with agents not mentioned in these protocols and 31 with discs containing amounts of the agent other than those recommended (Smith, in press). This paper will discuss only those breakpoints relevant to discs containing the recommended amounts of agents.

The majority of laboratories (22/25) used an SIR system and used two breakpoints, a minimum zone diameter for sensitive strains and a maximum for resistant strains, to categorise isolates. Three laboratories used an SR system and employed a single breakpoint to differentiate sensitive and resistant strains.

Twenty-two of the 25 laboratories reported that for any given antimicrobial agent they employed the same breakpoints for all the Group 1 organisms they examined. However, three laboratories divided these organisms. One reported different breakpoints for Gram-positive and Gram-negative bacteria. One divided Gram-negative Group 1 bacteria into three sub-groups (*Aeromonas salmonicida*, *Yersinia ruckeri* and *Vibrio* spp.) and reported different breakpoints for each sub-group (Table 1).

Table 2
Confidence associated with breakpoints analysed by agent

Agent	Percentage of reported breakpoints in each confidence category								
	AMX	ENR	ERY	FLO	FLU	OTC	OXA	SFT	Others
Breakpoints reported	13	10	7	16	18	28	19	27	18
Reasonable confident	62	50	43	50	44	68	47	63	56
Working hypothesis	38	50	29	38	50	32	47	37	33
Guess	0	0	29	12	6	0	5	0	6

Abbreviations of agent names as in Table 4.