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Critical analysis of available data on use of antibiotics in aquaculture

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Growth conditions for Vibrio spp

Standard CLSI susceptibility test protocols with appropriate Quality Control exist for bacterial species that can be tested on

unmodified Mueller-Hinton media

at 35° C in 16-24h

at 28° C in 24-28 h

Can halophilic *Vibrio* spp be tested under these conditions?

Search for papers that address the antimicrobial susceptibility of non-cholera *Vibrios*

A total of 182 papers were accessed that reported susceptibility testing of non-cholera *Vibrio* spp

Number of studies using Disc Diffusion	**150**
Number of studies using MIC	**50**
Number of studies using E-test	**7**

Vibrio alginolyticus	**72**
Vibrio anguillarum	**19**
Vibrio harveyi	**50**
Vibrio parahaemolyticus	**88**
Vibrio vulnificus	**27**
Vibrio Spp	**66**

Number of Papers	Country
35	India
21	China
13	Malaysia
11	Mexico, Taiwan
10	Italy
9	Tunisia
8	Spain
7	South Korea, USA
5	Brazil, Nigeria, Turkey
3	Bangladesh, Indonesia, Thailand
2	Denmark, Germany, Iran, Norway, Philippines, South Africa
1	Angola, Australia, Cameroon, Canada, Chile, Croatia, France, Greece, New Zealand, Poland, Romania, Saudi Arabia, Singapore, UK, USA, Vietnam

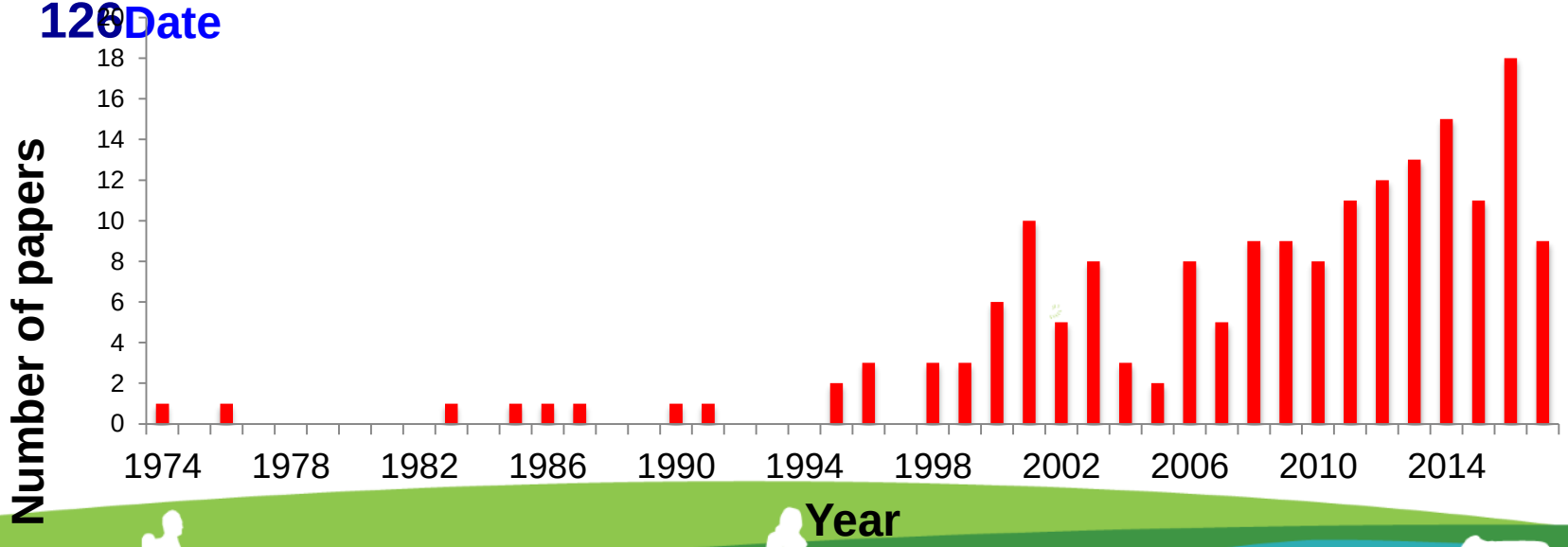
Citations

The 182 papers have received 6980 citation. The median citation per paper was 15

Aim

126 primarily aquatic animal health; 81 primarily human health

126 Date



Question 1

Vibrios are halophiles. Do they require addition of NaCl to Mueller - Hinton?

	Total studies	Number (%) with no added NaCl
V. alginolyticus	72	44 (63%)
V. anguillarum	19	10 (53%)
V. harveyi	50	27 (54%)
V. parahaemolyticus	88	61 (69%)
V. vulnificus	27	19 (70%)

All five species CAN be tested without additional NaCl

Question 2

Vibrios are aquatic organisms. Do they require incubation below 35° C?

	Studies performed at various temperatures		
	≥ 35° C	30° C	≤ 28° C
V. alginolyticus	**22 (47%)**	**11 (23%)**	**14 (30%)**
V. anguillarum	**3 (19%)**	**6 (38%)**	**7 (43%)**
V. harveyi	**11 (31%)**	**16 (46%)**	**8 (23%)**
V. parahaemolyticus	**26 (62%)**	**7 (17%)**	**9 (22%)**
V. vulnificus	**6 (38%)**	**6 (38%)**	**4 (25%)**

All five species CAN be tested at 35° C

How many studies were compatible with the recommendations of OIE Aquatic animal health code?

OIE Aquatic animal health code

1. Relevant authorities should perform studies to monitor and survey antimicrobial agent susceptibility of bacteria isolated from aquatic animals.

2. Studies should use standardised international testing protocols that have adequate internal quality controls

3. The meaning of any data should be established by application of internationally harmonised epidemiological cut-off values (ECVs)

4. Analysed data may be reported but raw unprocessed observed data must be reported

Question 3

In the studies from 38 countries is there a consensus as to the standard testing protocol that could be universally adopted?

source of susceptibility testing protocol	Number of studies
None given	103
CLSI guidelines (M2, M7, M45 M42, M49 or M100)	69
General ref to CLSI	6
Miscellaneous books or papers	29

There is a clear consensus in favour of the adoption of CLSI protocols

Disc studies

Of the 150 disc studies 51 (34%) stated that their testing method was based on standard CLSI protocols.

However of these,

14 studies modified the temperature and

16 used MH media with additional NaCl.

Only 31 (20%) of the disc studies used standard protocols published by CLSI

MIC studies

Of the 50 MIC studies 18 (36%) stated that their testing method was based on standard CLSI protocols.

However of these,

5 studies modified the temperature and

6 used MH media with additional NaCl.

Only 8 (16%) of the studies used the standard protocol published by CLSI

It would appear that in the 207 studies examined

we have 39 studies (31 disc and 8 MIC)

that have generated susceptibility data using a

standardised international testing protocol

81% of published studies did not use standard test protocols

OIE Aquatic animal health code

1. Relevant authorities should perform studies to monitor and survey antimicrobial agent susceptibility of bacteria isolated from aquatic animals.

2. Studies should use standardised international testing protocols that have adequate internal quality controls

3. The meaning of any data should be established by application of internationally harmonised epidemiological cut-off values (ECVs)

4. Analysed data may be reported but raw unprocessed observed data must be reported

Interpretive criteria

The meaning of susceptibility data can be established by application of epidemiological cut-off values (ECVs).

There are currently NO internationally accepted ECVs for *Vibrio* spp data generated using standardised test protocols.

ECVs are however very simple to establish

All that is needed is raw susceptibility data generated using a standard test protocol

OIE Aquatic animal health code

1. Relevant authorities should perform studies to monitor and survey antimicrobial agent susceptibility of bacteria isolated from aquatic animals.

2. Studies should use standardised international testing protocols that have adequate internal quality controls

3. The meaning of any data should be established by application of internationally harmonised epidemiological cut-off values (ECVs)

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Epidemiological cut-off values

In the 207 studies examined only 6 have made their raw data available

If raw unprocessed susceptibility data had been made available it would be simple to calculate relevant ECVs

We could generate these ECVs during the coffee break

Interpretive criteria – clinical breakponts

Standardised **clinical breakpoints** that can be applied to *Vibrio* spp MIC and disc zone data generated by tests performed using standard CLSI protocol (unmodified MH at 35˚ C) have been published in a variety of CLSI guidelines.

Recently these, together with breakpoints for other rarely isolated species (including *Aeromonas* spp), have been presented in the CLSI guideline M45-A3.

Application of CLSI clinical breakpoints

	Disc	MIC
All	**150**	**50**
Used standard test protocols	**31**	**8**
Used standard test protocols and clinical breakpoints	**16**	**3**

After examining 207 studies it would appear that we might have 19 (9%) studies where the data was generated using standard testing protocols and the meaning of the data can be understood by applying internationally harmonised criteria

Reservations concerning CLSI clinical breakpoints for *Vibrio* spp

There are two main reasons for suggesting that the M45-A3 clinical breakpoints should be applied with some degree of caution.

1. **There is a serious lack of empirical data justifying these breakpoints**

1. **The relevance of these breakpoints for studies concerning aquatic animal therapies is doubtful**

1. The breakpoints for *vibrio* spp given in M45-43 are of questionable validity

In the foreword to M45-A3 CLSI write

> "Users of the guideline should be aware that the very extensive microbiological, clinical, and pharmacodynamic databases normally used for setting breakpoints by CLSI **do not exist** for the collection of "orphan" organisms [such as *Vibrio* spp] described in this document."

In fact the breakpoints given in M45-A3 for *Vibrio* spp are just a copy of those given for all *Enterobacteriaceae* in M100- S27.

The same breakpoints are given for *Aeromonas* spp

These breakpoints are just 'best guesses' with no empirical support

2. Clinical breakpoints are host specific

Breakpoints aim to assess whether the concentration of an agent expected to be generated by the administration of a standard dose to a host is sufficient to be clinically successful in controlling an infection by a bacterium of a particular susceptibility in a particular host.

The value of a breakpoint is dependent on the agent's pharmacokinetics in the host species

Clinical breakpoints calculated for humans cannot be assumed to be appropriate in the context of infections of aquatic animals

CLSI clinical breakpoints should not be applied in a study being performed in the context of aquatic animal administrations

Application of CLSI clinical breakpoints

	Disc	MIC
All	**150**	**50**
Used standard test protocols	**31**	**8**
Used standard test protocols and clinical breakpoints	**16**	**3**
Used standard test protocols and clinical breakpoints relevant to their aim	11	0

Summary

207 published studies of the susceptibility of non-cholera *Vibrio* spp were examined

In only 11 studies were the data generated by internationally standardised test protocols and interpreted using relevant internationally proposed interpretive criteria (clinical breakpoints)

There are strong reasons for questioning the extent of the validity of the available clinical breakpoints as interpretive criteria

After a significant expenditure of time and money we have very little meaningful information

After a significant expenditure of time and money we have very little meaningful information

Where do we go from here?

OIE Aquatic animal health code

1. Relevant authorities should perform studies to monitor and survey antimicrobial agent susceptibility of bacteria isolated from aquatic animals.

2. Studies should use standardised international testing protocols that have adequate internal quality controls

3. The meaning of any data should be established by application of internationally harmonised epidemiological cut-off values (ECVs)

4. Analysed data may be reported but raw unprocessed observed data must be reported

Studies should use standardised international testing protocols that have adequate internal quality controls

ACTION

All future studies of *Vibrio* spp susceptibility should strictly follow the protocol in M45-A3

AND

All should perform and report the Quality Control procedures of the protocol

AND

All should report or make available their raw unprocessed data

The meaning of any data should be established by application of internationally harmonised epidemiological cut-off values

ACTION

Give up ALL attempts to use or set clinical breakpoints

BUT

Analyse the meaning of all data with epidemiological cut-off values calculated by statistically based methods such as NRI or ECOFFinder

The future

If we finally manage to generate some meaningful data where shall we put it?

Will we need an international agency to host a database of susceptibility data and epidemiological cut-off values for bacterial species relevant to aquaculture

Why were the studies performed?

Species	Primarily human health	Primarily aquatic animal health
All spp	81	126 (61%)
V. alginolyticus	24	48 (67%)
V. anguillarum	0	19 (100%)
V. harveyi	12	38 (76%)
V. parahaemolyticus	43	45 (51%)
V. vulnificus	12	25 (68%)