PRELIMINARY EVALUATION OF A SELECTIVE ISOLATION MEDIUM FOR *Vibrio hollisae*

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Introduction

Vibrio hollisae has been associated with diarrhoeal disease in humans[1,2]. Illness has been linked with the consumption of oysters[3]. Fresh isolates from clinical sources have been reported to show little or no growth on bile salts-containing media such as TCBS, the most widely used medium for the isolation of vibrios from clinical, food and environmental sources. The incidence of the organism in clinical samples and the environment is not known. The present work was undertaken to develop a medium for the isolation of this organism.

Two basic strategies were followed:

- Determine whether an antimicrobial agent could be identified to which *V. hollisae* strains were resistant such that this could be used as a selective agent
- Determine whether TSI agar, a bile salts-free medium developed for the isolation of vibrios from clinical sources[4], or a modification thereof, would be suitable for the isolation of *V. hollisae*.

Materials and methods

- Culture collection strains[5] were propagated on marine agar and stored as described previously[6]
- Marine agar 2216[7], TCBS[8] and Isosensitest Agar[8] were prepared according to the manufacturers' instructions. After sterilisation of the Isosensitest Agar, the medium was cooled to 50°C and 5% (v/v) laked horse blood added before pouring into plates
- TCI medium was prepared as described previously[4]
- *Vibrio hollisae* agar (VHA) was prepared as follows:
  - 55.1 g l^{-1} Marine agar 2216 was suspended in 6.1 g l^{-1} (0.05 mol l^{-1}) Tris-HCl buffer pH 7.6 containing 15 g l^{-1} potassium iodide and 72 mg l^{-1} water soluble phenol red
  - The medium was brought to the boil and then autoclaved at 121°C for 15 minutes
  - The medium was cooled to 50°C and 38 ml of 20% (w/v) D-mannitol and 38 ml of 20% (w/v) maltose were added, the medium mixed and poured into plates
- Inhibitory activity of antimicrobial agents was determined using a modified Stokes' disc diffusion test on ISA with laked blood using *Escherichia coli* NCTC 10418 as the control
- Estimation of growth on selective media was undertaken as follows:
  - A suspension of growth from marine agar was made in quarter strength Ringer's solution and further dilutions made in the same diluent
  - 0.1ml amounts of an appropriate dilution were spread over the surface of each of two tests plates and two marine agar plates
  - The plating efficiency were estimated as the percentage of the mean count on the selective agar (incubated for 24 h under the stated conditions) relative to the count obtained on marine agar at 30°C
  - Additional testing was undertaken using the modified ecometric technique[9].

Results

Sensitivity to antibiotics

- *V. hollisae* NCTC 11640 and NCTC 11641 were both sensitive to the following antibiotics (disc content):

Sensitivity to antibiotics			
Spectinomycin (10µg)	Amikacin (30µg)	Gentamicin (10µg)	Netilmycin (30µg)
Streptomycin (20µg)	Tobramycin (10µg)	Imipenem (10µg)	Cefaclor (30µg)
Cefuroxime (30µg)	Ceftazidime (30µg)	Cephradine (30µg)	Chloramphenicol (10µg)
Erythomycin (5µg)	Aztreonam (30µg)	Amoxycillin (2 µg)	Augmentin (30µg)
Methicillin (10µg)	Tetracycline (10µg)	Piperacillin (75µg)	Colistin (10µg)
Ciprofloxacin (5µg)	Nalidixic Acid (30µg)	Cotrimoxazole (25µg)	

- Both strains showed intermediate sensitivity to Pencillin G (1 unit) and Oxolinic acid (2 µg)
- Both strains were resistant to Fusidic Acid (10 µg), Teicoplanin (30 µg) and Vancomycin (30 µg).

Growth on selective media

- *V. hollisae* did not show growth on TCI medium if blood was omitted to enable the detection of fermentation
- *V. hollisae* grew on VHA as pink, non-fermenting colonies, 1.5 to 2 mm in diameter (see Figure 1)
- Other vibrios grew on VHA as yellow, fermenting colonies, 1.5 to 3 mm in diameter, depending on species (see Figure 2)
- Members of the *Enterobacteriaceae* showed weak or no growth on VHA
- *Pseudomonas aeruginosa* grew on VHA as small non-fermenting colonies (<0.5 mm)
- *Enterococcus faecium* colonies grew on VHA as pale yellow (weakly fermenting) colonies 1 mm in diameter
- Incubation under anaerobic conditions inhibited the growth of *P. aeruginosa* and increased the yellow colour of fermenting organisms
- Plating efficiencies on the selective media are shown in Table 1.

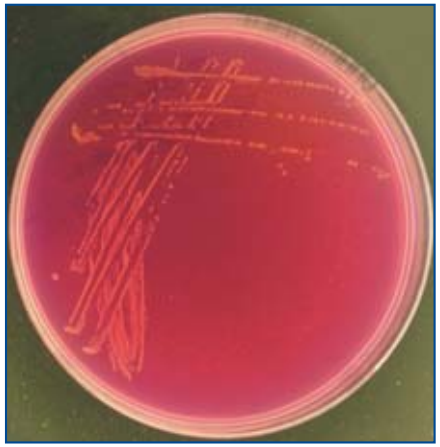

Figure 1: Appearance of *Vibrio hollisae* on VHA

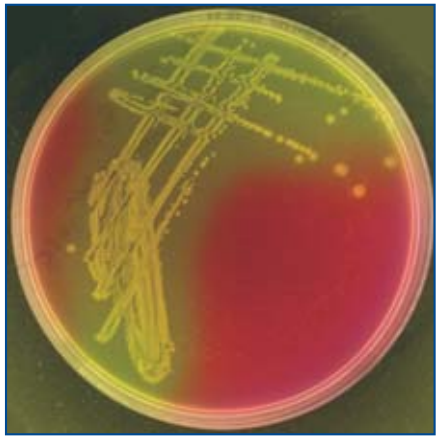

Figure 2. Appearance of *Vibrio cholerae* on VHA

Table 1: Plating efficiency of *Vibrio hollisae* and other bacteria on selective media

Strain	Species	Plating efficiency[1] on TCBS	VHA 37°C aerobic	VHA 37°C anaerobic
NCTC 11640	*Vibrio hollisae*	5	63	55
NCTC 11641	*Vibrio hollisae*	32	79	85
NCTC 10675	*Vibrio alginolyticus*	145	75	48
NCTC 4716	*Vibrio cholerae*	69	101	86
NCTC 10885	*Vibrio parahaemolyticus*	12	44	ND[2]
NCTC 12205	*Vibrio parahaemolyticus*	29	102	61
NCTC 7171	*Enterococcus faecium*	0	105	88
NCTC 9001	*Escherichia coli*	0	0	ND
NCTC 10332	*Pseudomonas aeruginosa*	0	97	0

1. Relative to the count on marine agar incubated aerobically at 30°C
2. Not done

Discussion

- None of the antibiotics to which the *V. hollisae* strains showed resistance was thought suitable for use as a primary selective agent for the isolation of this species
  - many other bacteria expected to be present in clinical and environmental samples would also be resistant
- Modification of TCI to produce a marine-agar based blood-free medium (VHA) allowed reasonable growth of *V. hollisae* strains and differentiation from other vibrios which fermented D-mannitol and/or maltose
- Anaerobic incubation of VHA may be used to suppress the growth of *P. aeruginosa*
- Vancomycin could be added to the medium to suppress the growth of Gram-positive bacteria.

Conclusions

VHA shows potential for use in the isolation of *V. hollisae* from clinical, food and environmental samples. Incubation under anaerobic conditions and the addition of vancomycin may be necessary to suppress the growth of some bacterial species commonly found in such samples. There is a need for the medium to be tested further with additional target and non-target strains.

References

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