Quantitative Recovery of *Aeromonas hydrophila* from Nsukka Sewage

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Abstract

Sewage samples were analyzed for the recovery of Aeromonas hydrophila. The pH of the samples ranged from 8.2 - 9.6 and the temperature from 20.0 – 28.7. Samples were enriched in alkaline peptone water medium (pH 8.4) before plating on different selective media. Media tested for the recovery of Aeromonas hydrophila from the sewage did not restrict the growth of other Aeromonas colonies, Vibrio spp and Pseudomonas spp. A. hydrophila had a recovery rate of 35.3%, 44.4%, 10.7% and 9.6% on starch-ampicillin agar, sheep blood-ampicillin agar, trypticase soy agar and thiosulphate citrate bile-sucrose agar respectively. Seasonal variations in temperature affected the rate of isolation of Aeromonas hydrophila from the sewage with higher microbial numbers occurring during the warmer months. Incorporation of ampicillin to a concentration of 10µg/mL in both starch- ampicillin agar and sheep blood-ampicillin agar improved the selectivity and sensitivity of A. hydrophila on these media. Media without ampicillin did not give good selectivity and specificity for A. hydrophila and were therefore not recommended for preliminary isolation of this organism from sewage samples.

Keywords: *Aeromonas hydrophila,* Recovery rate, Selective media, Sewage

Introduction

Aeromonas hydrophila is a bacterium which belongs to the family Aeromonadaceae and is indigenous to water ecosystems where it can multiply under appropriate conditions of temperature, nutrient concentration etc (Rippey and Cabelli, 1979). *Aeromonas* species have received increasing attention due to the epidemiological evidence which suggested that these organisms cause human diarrheal diseases (Deodhar *et al*., 1991; Kirov, 1993) and a variety of systemic and localized diseases in different mammals, reptiles and fish (Okrend *et al*., 1987; Schmidt *et al*., 2000).

Aeromonas species are ubiquitous in aquatic environments and readily isolated from both nutrient-rich and nutrient poor environments (Araujo *et al.,* 1991; Stechini and Domenis, 1994). These organisms have been isolated from such natural environments as soil, fresh, brackish, marine, waste water and sewage effluents (Araujo *et al.,* 1990). Sewage is rich in organic matter and this may result in substantially greater populations of these organisms and may also affect their distribution.

Only selective and differential media are required for *Aeromonas* detection and quantification. There are a few selective media for the recovery of *Aeromonas hydrophila* from natural environments. The choice of a medium should be based on its specificity and selectivity with the suppression of background microorganisms. This study was undertaken to evaluate the influence of some selective media and some environmental parameters on the rate of recovery of *Aeromonas hydrophila* from sewage samples.

Materials and Methods

Sample collection: Sewage samples (250 mL each) from the University of Nigeria Sewage Treatment Plant were collected into 500 ml sterile glass conical flasks. The samples were collected twice monthly from the superficial layers (about 35 cm from the surface). The pH and temperature were determined at the point of collection using digital pH meter (pH–500, Comecta, S.A.) and a thermometer, (Zeal, Made in England) respectively. The samples were taken to the laboratory where they were processed within 2 h of collection.

Media: Four media were tested for the isolation of *Aeromonas hydrophila* from sewage samples: trypticase soy agar (BBL Microbiology Systems, Cockeysville, USA); thiosulphate citrate bile-sucrose (TCBS) agar (Oxoid, UK). Starch-ampicillin agar was prepared according to Palumbo *et al.* (1985a), sheep blood-ampicillin agar was prepared according to the method of Kay *et al.* (1985).

Isolation of bacteria: Sewage samples (1 mL each) were added into conical flasks containing 9 mL of alkaline peptone water diluents (pH 8.4). The samples were incubated at 120 rpm in a Gallenkamp Orbital Incubator for 24 hours at 28^0C. Serial dilutions of the samples were done according to the method described by Collins and Lyne (1970). Aliquots (0.1 ml) of samples were inoculated onto selective agar plates in triplicates. The bacteria were identified by microscopic examination and by biochemical and physiological tests. The fermentation patterns of carbohydrates were

determined with reference to Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

Statistical Analysis: Analysis of variance (ANOVA) was used to determine the differences in quantitative growth of the isolates on different selective media tested.

Results

The characteristics of *A. hydrophila* from Nsukka Sewage is presented on Table 1. Several media were compared for their ability to allow for quantitative recovery of *A. hydrophila* from sewage samples (Table 2).

Table 1: Characteristics of *A. hydrophila* isolate

Test	Result
Gram reaction	-
Shape	Rod
Growth in nutrient broth with 1% NaCl	+
Growth in nutrient broth at 37% ^{o}C	+
Spore production	-
Motility	+
Catalase	+
Urea hydrolysis	-
Nitrate reduction	+
Citrate utilization	+
Gelatin liquefaction	+
Casein hydrolysis	±
Methyl red	+
Indole production	+
Voges-Proskauer reaction	+
Oxidase test	+
Haemolysis of sleep blood agar	+
Fermentation of:	
Fructose	AG
Glucose	AG
Galactose	Ag
Lactose	Ag
Maltose	Ag
Mannose	Ag
Sucrose	AG
Glycerol	A
Mannitol	AG
Arabinose	AG
Xylose	G
Sorbitol	-
Rhamnose	-
Raffinose	-

A= acid production; G= Gas production, ¼ or more volume of Durhams tube; g=gas less than ¼ volume of Durham's tube; ± variable reaction; + positive reaction; - negative reaction

Table 2: Comparison of several media for the recovery of *A. hydrophila* from sewage samples

Medium	Number of samples	Positive Samples (%)
SAA	24	35.3
SBAA	24	44.4
TSA	24	10.7
TCBS	24	9.6

Percent recovery was determined based on the number of counts positive for *A. hydrophila*. Analysis of variance was performed and the recovery rates on different selective media tested. There was a significant ($P < 0.05$) difference in the ability of the media to allow for quantitative recovery of *A. hydrophila* and also to allow for easy differentiation of *A. hydrophila* from background microflora.

The population levels of *A. hydrophila* isolated from the sewage throughout the sampling period are given in Table 3. Changes in pH did not result in significant changes in the numbers of the isolate recovered during the sampling period. The variations in temperature affected the population levels of the isolates on different media with the highest viable counts obtained during the warmer months with an average temperature of 27.9^{o}C and lowest counts obtained during the colder months with mean temperature of 22.0^{o}C. Total viable counts from selective media tested also showed variations at the same temperature and pH. Media containing ampicillin gave higher counts than media without ampicillin (Table 3).

Discussion

Sewage samples were enriched in an alkaline peptone water medium and shaken in an orbital shaker before preliminary isolation. Kay *et al.,* (1985) found that enrichment in alkaline peptone water before isolation of *A. hydrophila* from stool samples resulted in increased isolation rate. Orlob (1956) conducted an experiment in which two sewage samples were analyzed. One sample was shaken, the other was not. The former showed an initial increase in bacterial numbers whereas the latter demonstrated an abrupt decrease in bacterial numbers and then remained about 13% of the formers population for the rest of the experiment. This study showed that pre enrichment of sewage sample in alkaline peptone water (pH 8.4) followed by incubation in an orbital shaker at 28^{O}C for 24 h was very successful for the recovery of *A. hydrophila*.

Comparison of different selective media for the isolation of *A. hydrophila* showed a significant ($P < 0.05$) difference on the recovery rates. The recovery rate on starch -ampicillin agar was 35.3% while with trypticase soy agar only 10.7% was achieved. The most effective medium for recovery of *A. hydrophila* from sewage samples was sheep-blood ampicillin agar with a recovery rate of 44.4%, while the least effective in detection and recovery of *A. hydrophila* was thiosulphate citrate bile sucrose agar with recovery rate of 9.6%. The need to quantitatively recover *A. hydrophila* led to attempts at incorporating an agent that would prevent the growth of *Vibrio* spp and members of the family Enterobacteriaceae which are always present in similar environments (Golas *et al*., 2002). Several different media have been used to isolate *Aeromonas* from environmental samples: m-aeromonas agar (Rippey and Cabelli, 1979), pril-xylose ampicillin agar (Rogol *et al.,* 1979), starch-ampicillin agar (Palumbo *et al*., 1985a), ampicillin-dextrin agar (Havelaar *et al.,* 1987) and SCAP-10C agar (Huguet and Ribas 1991). All these media contain ampicillin. Ampicillin was chosen for adequate suppression of the background micro-flora as it is a selective agent with a broad spectrum

Table 3: Distribution of Aeromonas *hydrophila* in relation to the month of sampling, pH, temperature and media

Month of sampling	Total viable counts (mean CFU/mL)					
	pH	Temperature	TCBS	SBAA	TSA	SAA
January	9.3	27.2	$5.2x10^6$	$3.4x10^9$	$1.9x10^5$	$8.7x10^8$
February	8.2	28.0	$1.0x10^5$	$1.3x10^9$	$1.5x10^6$	$3.5x10^9$
March	8.6	25.7	$2.8x10^4$	$3.2x10^8$	$5.1x10^5$	$6.5x10^7$
April	8.3	23.0	$8.2x10^3$	$4.7x10^7$	$4.5x10^4$	$7.4x10^7$
May	9.6	20.0	$1.7x10^2$	$2.3x10^6$	$8.2x10^2$	$1.3x10^4$
June	8.4	21.0	$1.2x10^3$	$4.3x10^5$	$6.1x10^2$	$2.1x10^6$
July	8.2	20.3	$1.1x10^3$	$1.9x10^6$	$5.0x10^3$	$1.7x10^5$
August	8.5	24.0	$3.0x10^3$	$1.3x10^7$	$6.5x10^4$	$5.1x10^6$
September	8.7	26.7	$3.2x10^3$	$4.5x10^7$	$1.6x10^5$	$3.6x10^8$
October	8.4	27.7	$5.6x10^4$	$8.3x10^7$	$7.6x10^5$	$2.2x10^7$
November	8.3	27.7	$5.0x10^5$	$7.6x10^7$	$2.7x10^6$	$8.7x10^8$
December	8.8	28.7	$2.4x10^5$	$7.4x10^9$	$4.6x10^6$	$6.4x10^9$

activity. Havelaar *et al.,* (1987) showed that the effect of ampicillin on *Aeromonas* is generally negligible. From this study, incorporation of ampicillin to a concentration of 10 µg/ml improved the recovery of *A. hydrophila* from sewage samples as compared with media without ampicillin (Table 2). Media without ampicillin were found not to be very suitable for isolating *Aeromonas hydrophila* from sewage samples because they showed little specificity and selectivity.

The result in Table 3 gives the viable counts of *A. hydrophila* recovered from sewage samples. Highest counts were obtained during the warmer months may be due to increases in temperature of the sewage, while lower counts were obtained during the colder months. This result is in agreement with the findings of Burke *et al.,* (1984) who also found seasonal variations in the frequency of isolation of *A. hydrophila* from a metropolitan water supply. A similar finding was reported by Monfort and Baleux (1990) who observed an increase in the population of *A. hydrophila* in the summer months when pond temperatures were more than 20^oC and a decrease in the winter when pond temperatures were lower. Other studies have shown that the levels of *Aeromonas* spp are strongly correlated with temperatures. Biamon and Hazen (1983) reported that temperature was the most important factor responsible for higher population density of *Aeonomas hydrophila* in coastal waters. Such observations have been confirmed by investigators studying an estuary microenvironment (Kaper *et al.,* 1981). Although, Hazen *et al.,* (1978) investigating the prevalence of *Aeromonas* species in water samples found that temperature was not significantly related to the distribution of *A hydrophila.* From this study, the population of *A. hydrophila* was highest in the warmer months and lowest in the colder months. Therefore, recovery of *A. hydrophila* from the sewage was dependent on the environmental temperature.

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