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Biochemical Characteristics and Virulence Factors of Nigerian Strains of *Aeromonas* species Isolated from Cases of Diarrhoea and Domestic Water

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A total of 40 isolates of *Aeromonas* inclusive 12 from cases of diarrhea and 28 from water samples comprising 24 *A. hydrophila,* 10 *A. sobria* and 6 *A. caviae* were subjected to analysis of their biochemical characteristics and virulence factors. Fermentation of mannitol, lactose and indole was observed in all the 14 species of *A. hydrophila* recovered from water samples. All the 2 clinical isolates of *A. sobria* tested fermented mannitol and lactose. Among the virulence factors tested, 57.54% of the environmental isolates produced b-hemolysin, while higher 66.66% of the clinical isolates produced b-hemolysin. There was significant difference between clinical (92.85%) and environmental (66.66%) isolates of *Aeromonas* with respect to their enterotoxigenicity in suckling mice in-vivo. The result suggests that all the haemolytic environmental isolates could be enteropathogenic. From this study, we were able to delineate the difference between clinical and environmental isolates of the Nigerian strains of *Aeromonas* with respect to their biochemical characteristics, virulence factors and enterotoxigenicity. Further studies involving serogrouping, cytotoxic studies and molecular typing of aeromonads in Nigeria is necessary to reveal other virulent factors involved in diarrhoea.

Key words: Biochemical characteristics, clinical and environmental Virulence factors.

Aeromonas species are ubiquitous, gram- negative, non spore forming, motile or, non-motile, aerobic or facultatively anaerobic rod or coccobacillary curved rods belonging to the bacterial genus *Aeromonas* and family *Vibrionaceae* (Cipriano and Bullock, 2001). The motile mesophilic *Aeromonas* species are primarily organisms of aquatic and terrestrial environment. The organisms occur in fresh, estuarine and coastal water bodies and in both chlorinated and non chlorinated water (Ciprino and Bullock, 2001). Aeromonads have been associated with human cases of diarrhea especially in children, aged individuals and in immunocompromised patients (Gracey *et al.,*

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1982). Incidence of *Aeromonas* gastroenteritis has been reported world over and its incidence in developed countries has been reported to be relatively low compared to that in the developing countries. In Nigeria, *Aeromonas* diarrhea has been reported from Port Harcourt, Lake Chad, Kainji, Vom and Zaria (Okpokwasili and Ogbulie 2001). Several molecular typing techniques have been employed for typing of *Aeromonas* species and these have indicated that the clinical and environmental aeromonads are different. *Aeromonas* from human diarrhoeic stools and drinking water samples were reported to be dissimilar by biotyping and ribotyping in conjunction with gas liquid chromatography of cell wall fatty acid methyl esters. Studies by restriction enzyme fingerprinting by Alvandi and Anathan (2003) indicated that there were differences between clinical and environmental strains of *Aeromonas* species; and that only some strains occurring in water are potentially enteropathogenic. In order to find out the differences between the Nigerian strains of clinical and environmental *Aeromonas* species, we analyzed the biochemical characteristics and virulence factors.

Methodology

Fourty isolates sourced from bacterial zoonosis laboratory,ABU Zaria were subjected to biochemical characterization using standard protocols (Janda *et al.,* 1984). Production of β-hemolysin was determined by conventional inoculation on 10% sheep blood agar. Presence of large zone of β-hemolysis in and around the colonies were determined according to standard protocols (Burke *et al*., 1991). *Aeromonas* isolates were tested for enterotoxigenicity *in vivo* in 3-day old sucking mice (Burke *et al.,* 1981).

RESULTS

Biochemical Characteristics

All the 24 (60.0%) *A.hydrophila* isolates10(25%) of *A.sobria* and 6(15%) of *A.caviae* were aerogenic (Table 1) . 20% of the clinical isolates fermented lactose while 70% of the environmental isolates do not ferment lactose. 30% of the clinical isolates fermented mannitol while 62.5% from domestic water samples fermented mannitol. Fermentation of arabinose was observed in 15% of the isolates from clinical sources, and the feature was also associated with 30% of *Aeromonas* from water samples. All the *Aeromonas* strains encountered in this studies donot ferment salicin. Production of acetyl methyl carbinol was observed in 25% of the clinical samples and 50% of the isolates from water, and was usually associated with *A.hydrophila* and *A.*

Table 1. Biochemical Features of *Aeromonas* Species (n=40)

Test	Percentage positive							
	A. hydrophila		*A. sobria*		*A. caviae*		Total	
	C	E	C	E	C	E	C	E
Number of isolates tested	6	14	2	8	4	6	12	28
Gas production from TSI	2	8	2	0	0	1	4	9
Fermentation of lactose	2	14	2	8	4	6	8	28
Fermentation of mannitol	6	14	2	8	4	3	12	25
Acid from arabinose	1	6	1	3	4	3	6	12
Fermentation of salicin	0	0	0	0	0	0	0	0
Production of indole	6	14	4	6	0	0	10	20
Voges-proskaeur test	4	6	1	7	4	6	9	19
Aesculin hydrolysis	4	10	0	0	2	4	6	14
Lysine decarboxylase	5	10	2	6	4	4	11	20
Arginine dihydrolase	0	0	1	2	3	4	4	6
Amylases Lipases CAMP (cyclic adenosine monophosphate) like factor	405	213	100	230	010	150	515	583

C: = Clinical E: = Environmental

sobria, while none of *A.caviae* isolates produced acetyl methyl carbinol. Aesculin hydrolysis was observed in 15% of the clinical isolates and 35% of the isolates from domestic water samples, and this trait was found to be associated with *A.hydrophila* and *A.caviae*

Virulence factors

Majority of the clinical isolates (66.66%) produced enterotoxins, while a relatively lower percentage (57.54%) of aeromonads recovered from water samples produced β-hemolysin (Table 2).

There is significant difference between the clinical and environmental *Aeromonas* isolates with respect to enterotoxigenicity in suckling mice. 92.85% of the *Aeromonas* isolates of clinical origin were found to be enterotoxigenic while only 66.66% of the environmental were enterotoxigenic by sucking mouse assay.

Table 2. Virulence factors of clinical (C) and environmental (E) *Aeromonas* isolates

Species	Hemolysin		Sucking mice	
	C	E	C	E
A. hydrophila n=24	1(4)	1(2)	4(4)	2(3)
A. sobria n=10	2(2)	1(2)	3(4)	2(3)
A. caviae n=6	3(3)	2(3)	6(6)	2(3)
Total 40	6(9)	4(7)	13(14)	6(9)
Percentage	66.66%	57.54%	92.85%	66.66%

Figures in parenthesis are number of isolates tested. n=Total number of isolates examined

DISCUSSION

Biochemical characteristics

The findings from the Nigerian *Aeromonas* strains subjected to biochemical characterization indicates that all the *A.hydrophila, A.sobria* and *A. caviae* encountered were aerogenic. Production of acetoin was confined to *A.hydrophila* and *A.sobria.* Biochemical characteristics such as fermentation of arabinose, lactose, esculin hydrolysis, and lack of acid production from salicin were found to deviate from the ideal phenol type for each isolate Janda *et al.* (1984) have earlier reported similar deviation in biochemical characteristics from ideal phenotypes (Gracey *et al.,* 1982). 70% of *Aeronomas* isolates obtained from domestic water samples, mainly comprising *A .hydrophila*, fermented lactose. This facts needs to be considered when selective isolation were not employed for isolation of aeromonads from clinical and environmental samples. The present study indicated that, From the 15% of *Aeromonas* isolates of clinical sources that fermented arabinose, majority were *A.caviae.* In an earlier study from Australia, a relatively higher percentage (18%) of the aeromonads were reported to be able to ferment arabinose (Janda and Bottone, 1984). Decarboxylation of lysine associated with clinical *Aeromonas* indicated that *A.hydrophila* was (12.5%) and *A.sobria* (5%) while *A.caviae* (10%) . This result corroborates well with those reported earlier (Burke *et al.,* 1984). Production of CAMP like factor from this finding is usually associated with hemolytic strains of *A. hydrophila.* Hence the proposal of Figura and Gugliemetti (1987) that the motile mesophilic *Aeromonas* strains can be presumptively differentiated based on these trait appear to be applicable (Alvandi and Ananthan, 2003).

Virulence factors

Among the *Aeromonas* isolates obtained in the present study, 66.66% of the clinical isolates were hemolytic, while more than 57.54% of those obtained from environmental sources were able to hemolyse 5% sheep erythrocytes. Some studies indicated that hemolysin production in a relatively lesser percentage isolates compared to the results

obtained in this study (Sighn *et al.,* 1992), majority of *A.sobria* isolated in the present study were hemolytic. Out of the 9 isolates from water samples tested for enteropathogenicity by suckling mouse assay, (66.66%) of them showed their ability to induce enterotoxigenic response in the suckling mice *in-vivo*. It has been reported that enteropathogenicity of environmental isolates was low compared to those from clinical source (92.85%) by suckling mouse assay.

CONCLUSION

Analysis of the *Aeromonas* isolates revealed some differences between isolates from clinical and environmental sources. The notion that the *Aeromonas* species are always non-lactose fermenters may prove wrong and selective media such as Inositol Brilliant green Bile salts (IBB) agar or Cefsulodin Irgasan Novobiocin (CIN) agar and Starch Ampicillin (SA) agar must be incorporated with enteric media for the recovery of *Aeromonas* species. Further studies involving serogrouping, cytotoxic studies and molecular typing of the *Aeromonas* species could play a role to determine certain factors responsible for diarrhoea in Nigeria.

REFERENCES

1. Alvandi, S.V. and Ananthan, S., Biochemical characteristics, serogroups, and virulence factors of *Aeromonas* species isolated from cases of diarrhea and domestic water samples in chennai. *Journal of Clinical and Environmental Microbiology,* 2003; **23**: 132-134.
2. Burke, V. Robinson, J. Berry, R.J. and Gracey, M., Detection of enterotoxins of *Aeromonas hydrophila* by a suckling – mouse test. *Journal of Medical Microbiology,* 1981; **14**: 401-408.
3. Cipriano, R.C. and Bullock, G.L., Furuclosis and other diseases caused by *Aeromonas salmonicida. Journal of fish Health Research,* 2001; **16**: 21-32.
4. Figura, N. and Guliemetti, P., Differentiation of motile and non mesophilic *Aeromonas strains* into species by testing for a CAMP-like factor. *Journal of clinical microbiology*, 1987; **25**: 1341-9342.
5. Gracey, M., Burke, V. and Robinson, J., *Aeromonas* associated gastroenteritis. *Lancet,* 1982; **2**: 1304-1306.
6. Janda, J.M., Reitano, M. and Bottone, E.J., Biotyping of *Aeromonas* isolates as a correlate to delineating a species – associated disease spatrum. *Journal of Clinical Microbiology,* 1984; **47**: 116-1149.
7. Okpokwasili, G.C. and Ogbulie, J.N. 92001). The Biology and seasonality of Tilapia (*Oreochromis niloticus)* brown – patch syndrome. *Journal of Food Protection,* **50**: 509-513.
8. Sighn, D.V. and Sanyal, S.C., Hemolysin and enterotoxin production by *Aeromonas caviae* isolated from diarrhoeal patients, fish, and environment. *Journal of Diarrhoeal Disease Research,* 1992; **10**: 16-20.