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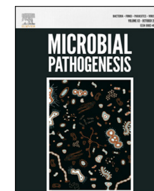

Pathogenic potentials of *Aeromonas* species isolated from aquaculture and abattoir environments

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ABSTRACT

The present study elucidated the presence of antibiotics resistance, virulence genes and biofilm potentials among *Aeromonas* species isolated from abattoir and aquaculture environments in Benin City, Nigeria. A total of 144 wastewater samples were obtained from two independent aquaculture and abattoir environments between May and October 2016. *Aeromonas* species were isolated on Glutamate Starch Phenol Red (GSP) agar and confirmed using API 20NE kits. Antimicrobial susceptibility profile of the isolates was carried out using standard disc diffusion assay while biofilm potentials were detected by the microtitre plate method and PCR technique was used to detect antibiotics resistance and virulence gene markers. Overall, 32 and 26 *Aeromonas* species were isolated from the abattoir and aquaculture environments respectively. Isolates from both environments were completely resistant (100%) to penicillin G, ertapenem and tetracycline; whereas aquaculture isolates exhibited absolute sensitivity (100%) towards cefepime. All the virulence gene markers (*aer*A, *hly*A, *alt*, *ast*, *laf*, *asc*F-G, *fla*, *lip*, *stx*1, and *stx*2) investigated in this study (except *laf*) were detected in isolates from both environments. The *laf* genes were only detected in isolates from abattoir environments. Antibiotics resistant genes including *pse*, bla_{TEM} and class 1 integron were detected in isolates from both environments. Majority of the isolates (53/58 91.4%) from both environments; demonstrated capacity for biofilm potential. The detection of antibiotic resistance and virulence gene markers as well as biofilm forming ability in *Aeromonas* species isolated from aquaculture and abattoir environments raise serious public health concern worthy of further investigation.

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1. Introduction

The continuous advancement of antibiotic resistance in pathogenic organisms is proportionate to the selective pressure of antibiotics and resulting in proliferation of antibiotic drug resistant strains [1,2]. It has been reported that microbial variations are constantly evolving; consequently resulting in the development of multidrug resistance due to the dissemination of antimicrobial agents [3,4]. Environmental pollution resulting from bacteria associated with antibiotic resistance and virulence genes can enhance the possibilities of human pathogenic bacterial strains to acquire and spread resistance and virulence determinants [5,6]. The discharge of effluents harbouring pathogenic microbiota enriched in resistant and virulent trait elements into the surrounding environments increases the likelihood of possible pathogens to acquire novel antibiotic resistance and virulence genes [2,7,8]. The dissemination of resistance and virulence genes in ecosystems can dare the resident dynamics, density and physiology of the microbial diversity [9,10].

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Aeromonas species are Gram-negative, non-spore-forming, facultative anaerobic, rod-shaped, mesophilic, water- and food-borne bacteria that are well-thought-out to be zoonotic human pathogens [11–13]. They have been implicated as a significant cause of severe dysentery, bacteremia, and diarrhoea [14–16]. In veterinary and human medicine, *Aeromonas* strains have been reported as opportunistic pathogen and have been isolated from wound, and fecal specimens [14,16–18]. Detection of *Aeromonas* species in food producing animals indicates a potential health risk [19]. Cumulative data significantly claims that food animals are reservoirs of pathogenic bacteria and thus a potential source of human exposure [5,12,20].

Aeromonas species have been reported as agents of fish infection [12,20,21] and are connected with wound infections and diarrheal diseases in humans which may emanate due to contact with polluted water [1,6,14,17,22]. Resulting wound infections may thus become deep and systemic [23]. *Aeromonas* have also been implicated in eye, respiratory tract, sepsis, and other deep-seated infections [9,11]. In aquaculture and abattoir ecosystems, *Aeromonas* can cause bacterial infections which may result in relatively high antimicrobial resistance, promoting difficult to treat infections [1]. Treatment of ailment resulting from *Aeromonas* infection is habitually with the application of antibiotics. Due to the abuse of antimicrobial agents in food animals and aquaculture as growth promoters, therapeutic and prophylactic agents as well as in the community, multidrug resistant strains have emanated resulting in difficult to treat infections [5].

The distribution of *Aeromonas* in aquaculture and abattoir environments, its emerging problems as a resistant reservoir in such environment, and the tenacity to spread resistance and virulence determinants in the environment are all of public health concern. The capacity of *Aeromonas* species to resist multiple-drug is also a consequence of their ability to acquire new antibiotic resistance and virulence genes [9,17]. Mobile genetic elements referred to as integrons define a point-specific recombination organisation that is accountable for its capacity to acquire many antibiotic resistance genes [3,9,20,24].

The capacity of *Aeromonas* spp. to initiate an infection is multifactorial and multifaceted. It may include expression or secretion of a number of different virulent determinants acting collectively or independently [7,19,22]. The most prevalent ones are *hly*A and *aer*A gene which expresses haemolysin and aerolysin toxins production respectively [16]. Aerolysin is a significant virulence determinant in annexation of epithelial cells and gastroenteritis [12,14]. The manifestation of *hly*A, *aer*A, *ast*, and *alt* determinants could be indicative of diarrhoea-related virulence [23]. Shiga toxins expressed by *stx*1 and *stx*2 determinants are significant virulence genes in the cause of hemorrhagic, gastroenteritis, hemolyticuremic syndrome, and hemorrhagic colitis [13]. The *asc*F determinant which expresses type III secretion system also plays a significant role in its pathogenicity [13]. Studies also revealed that *Aeromonas* species that harbours sideway flagella as well as the flagellin gene (*fla*) are reportedly connected to dysenteric or persistent infections [24,25].

Bacterial attachment to solid surfaces is one of the crucial mechanisms resulting in the formation of biofilm [26]. It is a significant bacteriological incident in human and veterinary medicine as well as the environment [27,28]. *Aeromonas hydrophila* have been reported to attach to solid surfaces and form biofilms on glass surface, polystyrene, polyvinyl chloride, and stainless steel [16]. *Aeromonas* have also been reported to produce biofilms in aquatic milieu [24]. Biofilm is an undesirable growth of combined bacterial micro-colonies on exteriors deep-rooted in polysaccharide extracellular matrix [29]. Biofilm production have been reported to contribute to persistence of infections, due to its enhancement of antimicrobial resistance [7,30].

Previous studies have characterized *Aeromonas* species from humans and animals in Turkey [13]; two South African Rivers [24]; treated wastewater effluent and receiving surface water in Durban, South Africa [31]; poultry, cattle and pigs in South Africa [5,19]; cultured *Mugil capito* fish in Ismailia Governorate, Egypt [21]; and from clinical, food and environmental sources in Barcelona, Spain [16]. The objective of this study was to characterize *Aeromonas* species isolated from aquaculture and abattoir environments for presence of antimicrobial resistance, virulence determinants and biofilm formation potentials.

2. Materials and methods

2.1. Sample collection

A total of seventy-two (72) wastewater samples from two (2) abattoirs were obtained from different points at the abattoir such as the drains; stagnant water in pot holes around the abattoir environment where the meat is usually chopped and from water used to wash the chopped meat slaughtered at the abattoir every fourthnight in Benin City, Nigeria between May and October 2016. A total of seventy-two (72) wastewater samples were also collected from two (2) aquaculture environments in Benin metropolis within the same time frame. All samples were collected with the aid of sterile glass containers and transported in ice pack to the Applied Microbial Processes & Environmental Health Research Group (AMPEHRG) Laboratory in University of Benin, Benin City, Nigeria and analysed within 4 h after collection.

2.2. Isolation of the Aeromonas species

All samples obtained were serially diluted (10^1-10^5). The diluents were then pre-enriched by adding 100 μL from each diluent to test tubes containing 5 mL of Tryptone Soy Broth (Merck, Germany) and incubated at 37 °C for 18 h. After incubation, 100 μL of each of the incubated enriched samples were spread-platted on Glutamate Starch Phenol Red (GSP) agar base (Merck, Germany) which is selective for *Pseudomonas* (pink colonies) and *Aeromonas* (yellow colonies) species. The plates were incubated at 37 °C for 18–24 h. After incubation, yellow colonies were streak-platted on another GSP agar and incubated at 37 °C for 18 h to obtain discrete colonies. Purified isolates were then cultured on Tryptone Soy Agar (Merck, Germany) and thereafter stored on Brain Heart infusion broth at −20 °C with 30% glycerol.

2.3. Identification of the Aeromonas species

All Gram-negative, rod shaped, flagellated, oxidase-positive (secured with oxidase strip), yellow colonies on GSP agar were subjected to biochemical characterization using Analytical Profile Index (API) 20NE (BioMerieux, Marcy-l'Étoile, France) following the manufacturers instruction. *Aeromonas hydrophila* ATCC 7966 strain was used as positive control. The resultant API strips were critically studied and identities of the isolates were confirmed by adopting analysis presented by the API lab plus software (BioMerieux, Marcy l'Etoile, France).

2.4. Gelatinase, haemolysin and protease assay

Gelatinase activity was assayed on Luria-Bertani (LB) agar containing gelatine (30 g/L). The plates were incubated for 24 h at 30 °C and thereafter kept at 4 °C for 5 h. The presence of unclear halos on the nearby colonies was regarded as positive for gelatinase production [32]. Haemolysin activity was detected by cultivating each

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