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Antimicrobial Resistance Profile Characterization of *Enterococcus* Species Isolated from Aquaculture Environment

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Abstract

Aquaculture environments can become reservoirs of antibiotic-resistant strains of faecal bacteria including enterococci which are capable of contributing to the spread of antimicrobial resistance in the marine environment. The aim of this study was to characterize the antimicrobial resistance profile of *Enterococcus* species from various aquacultures (fish ponds) in Benin City, Edo State. A total of 376 water samples were obtained from four different fish ponds between February and July, 2015 and were investigated for the presence of *Enterococcus* species using standard culture based methods. The antimicrobial susceptibility profiles of the isolates were determined by using Kirby-Bauer disc diffusion method. From the 376 water samples analyzed for the presence of enterococci isolates, 100 isolates each from *Enterococcus faecalis* and *Enterococcus faecium* were isolated from the four fish ponds investigated. The mean enterococal counts from the four fish ponds investigated ranged from $4\pm0.01 \times 10^2$ to $12\pm0.17\times10^2$ cfu/ml. Statistical analysis reveals that there was a significant difference observed in the mean enterococcal counts obtained from the four different fish ponds in the study ($p< 0.05$). The distribution of the enterococcal isolates in the study from February to July reveals that 50 isolates from each pond were characterized. Statistical analysis also reveals that there was a significant difference observed in the distribution of the enterococcal isolates from the fish ponds ($p< 0.05$). High level resistance was observed against six antibiotics used in the study such that 15 enterococcal isolates displayed marked resistance to the action of trimethoprim, chloramphenicol, erythromycin, oxytetracycline, sulfamethoxazole, and ciprofloxacin. Adherence to adequate and proper use of manure products and frequent discharge of water from fish pond will reduce the high level of antimicrobial resistance in *Enterococcus* species isolated from fish ponds and also reduce the potential risk to human health.

Keywords: Antimicrobial Resistance Profile; *Enterococcus* Species; Aquaculture Environment; Antibiotics

Introduction

The rapid emergence and dissemination of antibiotic-resistant microorganisms in the global ecosystem has been widely reported as a significant public health issue, thus there is concern on the future ability to treat multi-drug resistant infections. Contaminated ponds can become reservoirs of virulent and antibiotic-resistant strains of faecal bacteria [1], including enterococci [2], which are capable of transmitting resistance genes to other bacteria by horizontal gene transfer mechanisms, thus contributing to the spread of resistance genes into the marine environment. Aquaculture has been a growing activity for the last 30 years worldwide and has become one of the fastest developing sources of animal protein to humans and animals due to dwindling wild fish stocks around the world, in particular Nigeria [3]. The importance of fish cannot be overemphasized. Fish is a low fat food, a great source of protein, vitamins and minerals. Annual domestic fish supply in Nigeria stands at about 400,000 tons [4]. Accumulation of surplus antimicrobials and antimicrobial residues may occur when the ponds are only rarely emptied at the time of fish harvest. Such a build-up could establish selective pressure favoring selection and growth of antimicrobial-resistant bacteria [5].

Enterococci are Gram positive cocci that are part of the human and animal intestinal microflora and are used as faecal indicator bacteria for assessing potential risks for human health and monitoring recreational waters [6]. Due to the ability of Enterococci to transfer transposons (including conjugative transposons), resistance plasmids, and sex pheromone plasmids to a broad range of recipients. Effective water management in fish ponds is one of the important factors that contribute to the success of fish culture [7].

Materials and Methods

Sample Collection

The water samples were obtained from three different fish ponds (P1, P2 and P3) where there was a deliberate impute of 100mg/kg animal waste/manure, while samples were also obtained from a fish pond (P4) where there was no history of animal waste/manure application. The sampling period was between February and July, 2015 in Benin City, Edo State. A total of 376 water samples were obtained from the four different fish ponds investigated in the study. The size of the ponds varies between 0.8 and 1.2 hectare. The fish ponds were located in Ikpoba hill, Sapkonba and Iyekeogba Estate, Benin City. During each visit to the pond, water discharge sample were collected from the fish pond by using clean plastic syringes and tube and were transported to the laboratory for microbiological analysis in cold ice pack and analyzed within 24h after collection [1].

Isolation of Bacterial Isolates from Water Samples

All media used for this study were prepared according to the manufacturer's instruction. The media used in the study include Bile Esculin agar, Nutrient agar, and Mueller Hilton agar. Isolation of *Enterococcus* species was carried out as described by [8]. One millilitre (1 ml) of the water samples was transferred into a sterile test tube containing 9.0 ml of peptone broth. This process was repeated for other sterilized test tubes so that at the end, dilution of 10^{-1}, 10^{-2} and 10^{-3} dilutions were obtained. A 0.1 ml from 10^{-1}, 10^{-2} and 10^{-3} dilution was then plated out using the pour plate method on Bile Esculin agar plates [9]. The Bile Esculin agar plates were incubated at 37°C for 24 - 48 h, after incubation the colonies were counted and expressed in colony forming unit per millilitre (cfu/ml). Discrete colony were sub-cultured on freshly prepared Bile Esculin agar plates, incubated at 37°C for 24-48 h and stored on Nutrient agar slant at 4°C for further laboratory analysis.

Characterization and Identification of Bacterial Isolates

The total viable bacteria counts (TVBC) formed were counted using a colony counter. Characterization of isolates was carried out as described by [8]. Bacterial isolates were identified on the basis of cultural, morphological, biochemical and sugar fermentation tests such as glucose, lactose, arabinose, sucrose, maltose, and mannitol [10].

Antibiotic Susceptibility Screening

The enterococcal isolates that were positively identified using the culture based methods were subjected to antibiogram characterization [11] All the bacterial isolates were tested for resistance or sensitivity to different antibiotics using the standard disc diffusion method (Kirby Bauer test). For the disc diffusion assay, bacteria were grown between 18 and 24 h on Mueller-Hinton agar, harvested and then suspended in 0.85% sterile physiological saline solution adjusted to a 0.5 McFarland turbidity standard, corresponding to 10^8 cfu/ml. The inoculum was streaked on plates of Mueller-Hinton agar using a sterile cotton swab and impregnated with appropriate antibiotics [12]. The results were recorded after 24 h of incubation at 37°C. Commercially available antibiotics discs, obtained from Mast

Diagnostics, Merseyside, United Kingdom, were used to determine the resistance patterns of the isolates against 6 different antibiotics [13]. The diameter of the zone of inhibition around each disc was measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in accordance with the recommended standard established by the Clinical Laboratory Standards Institute [14]. All data in the study were analyzed using the statistical package (SPSS). P-values < 0.05 were considered statistically significant.

Results and Discussions

The mean enterococcal count in the study is presented in (Table 1). It was observed that the mean enterococcal counts from February to July for P1 ranged between 4±0.01 × 10^2 and 12±0.17 × 10^2 cfu/ml. A mean enterococcal count of 2±0.02 × 10^2 and 16±0.20 × 10^2 cfu/ml was observed in P2. For P3, a mean enterococcal count of 4±0.01 × 10^2 and 12±0.04 × 10^2 cfu/ml was observed while a mean enterococcal count of 2±0.00 × 10^2 and 16±0.13 × 10^2 cfu/ml was observed in P4. Statistical analysis reveals that there was a significant difference observed in the mean enterococcal counts ($p < 0.05$).

Months	Bacterial isolates (10^2 cfu/ml)				*P*-value
	Pond 1	Pond 2	Pond 3	Pond 4	
February	$10±0.01^B$	$12±0.02^A$	$14±0.03^B$	$12±0.00^A$	0.014
March	$16±0.00^B$	$12±0.05^A$	$20±0.02^C$	$18±0.02^{BC}$	0.032
April	$18±0.31^B$	$20±0.01^C$	$18±0.00^B$	$16±0.00^A$	0.002
May	$20±0.00^B$	$22±0.00^C$	$22±0.00^C$	$14±0.01^A$	0.015
June	$20±0.12^C$	$18±0.02^B$	$14±0.01^A$	$26±0.13^D$	0.032
July	$22±0.17^A$	$26±0.20^C$	$22±0.04^A$	$24±0.01^B$	0.001

Table 1: Mean *enterococcal* counts from the various ponds.

Values are means of triplicates ± standard deviations (SD). Mean differences are presented as A, B, C, and D across column and values with significant difference carry different alphabets.

The multidrug resistant profile of the isolates from the four fish ponds are presented in Table 2. A total of 89 of the enterococcal l isolates in the study were resistant to the action of sulfamethoxazole and oxytetracycline while 15 of the enterococcal isolates showed marked level of resistance to the action of sulfamethoxazole, oxytetracycline, chloramphenicol, trimethoprim, erythromycin, and ciprofloxacin. Statistical analysis reveals that there was a significant regression ($r = 0.876$) of the multidrug resistant profile on the number of isolates studied ($p > 0.05$). The distribution of the enterococcal isolates in the study from February to July is presented in Table 3. A total of 32 *E. faecalis* and 18 *E. faecium* were isolated from P1. A total of 20 *E. faecalis* and 30 *E. faecium* were isolated from P2. A total of 12 *E. faecalis* and 38 *E. faecium* were isolated from P3. A total of 40 *E. faecalis* and 10 *E. faecium* were isolated from P4. Statistical analysis reveals that there was a significant difference observed in the distribution of the enterococcal isolates from the fish ponds ($p < 0.05$). Antimicrobial resistance profile of the enterococcal isolates from the fish ponds are presented in Table 4. A total of 25/200(12.50%) of the enterococcal isolates were resistant to the action of trimethoprim; 21/200 (10.50%) of the enterococcal isolates were resistant to the action of chloramphenicol; 39/200 (19.50%) of the enterococcal isolates were resistant to the action of erythromycin; 15/200 (7.50%) of the enterococcal l isolates were resistant to the action of oxytetracycline; 9/200 (4.50%) of the enterococcal isolates were resistant to the action of sulfamethoxazole; 89/200 (44.50%) of the enterococcal l isolates were resistant to the action of ciprofloxacin. Statistical analysis reveals that there was no significant difference observed in the antimicrobial resistance profile of the enterococcal isolates from the fish ponds (P> 0.05).

Multidrug resistance profile	Number of isolates n=200
SMX^R, OXY^R	89
SMX^R, OXY^R, CHL^R	39
SMX^R, OXY^R, CHL^R, TRI^R	25
SMX^R, OXY^R, CHL^R, TRI^R, ERY^R	21
SMX^R, OXY^R, CHL^R, TRI^R, ERY^R, CIP^R	15

Table 2: Multidrug resistance profile of the *Enterococcal* isolates from the fish ponds.

TRI: Trimethoprim; CHL: Chloramphenicol; ERY: Erythromycin; OXY: Oxytetracycline; SMX: Sulfamethoxazole; CIP: Ciprofloxacin.

Sampling Period	Pond 1		Pond 2		Pond 3		Pond 4		Total n=200	*P*-value
	E. faecalis n=32	*E. faecium* n=18	*E. faecalis* n=20	*E. faecium* n=30	*E. faecalis* n=12	*E. faecium* n=38	*E. faecalis* n=40	*E. faecium* n=10		
February	4 (12.50)	3 (16.67)	3 (15.00)	5 (16.67)	3 (25.00)	7 (18.42)	6 (15.00)	2 (20.00)	33 (16.50)	0
March	3 (9.38)	5 (27.78)	4 (20.00)	6 (20.00)	2 (16.67)	5 (13.16)	7 (17.50)	1 (10.00)	33 (16.50)	0.001
April	6 (18.75)	2 (11.11)	3 (15.00)	5 (16.67)	2 (16.67)	3 (7.90)	8 (20.00)	1 (10.00)	30 (15.00)	0.003
May	5 (15.63)	3 (16.67)	2 (10.00)	4 (13.33)	2 (16.67)	4 (10.53)	5 (12.50)	2 (20.00)	27 (13.50)	0
June	6 (18.75)	3 (16.67)	5 (25.00)	3 (10.00)	1 (8.33)	9 (23.68)	6 (15.00)	1 (10.00)	34 (17.00)	0.002
July	8 (25.00)	2 (11.11)	3 (15.00)	7 (23.33)	2 (16.67)	10 (26.32)	8 (20.00)	3 (30.00)	43 (21.50)	0.002

Table 3: Distribution of the *enterococcal* isolates from the fish ponds

Values in parenthesis represents (%)

Antibiotics	Pond 1		Pond 2		Pond 3		Pond 4		Total n=200	*P*-value
	E. faecalis n=32	*E. faecium* n=18	*E. faecalis* n=20	*E. faecium* n=30	*E. faecalis* n=12	*E. faecium* n=38	*E. faecalis* n=40	*E. faecium* n=10		
TRI	20 (62.50)	1 (5.56)	0 (0)	2 (6.67)	0 (0)	2 (5.26)	0 (0)	0 (0)	25 (12.50)	0.239
CHL	1 (3.13)	15 (83.33)	0 (0)	2 (6.67)	1 (8.33)	1 (2.63)	0 (0)	1 (10.00)	21 (10.50)	0.184
ERY	1 (3.13)	0 (0)	17 (85.00)	20 (66.67)	1 (8.33)	0 (0)	0 (0)	0 (0)	39 (19.50)	0.147
OXY	0 (0)	1 (5.56)	1 (5.00)	4 (13.33)	0 (0)	0 (0)	10 (25)	1 (10.00)	15 (7.50)	0.124
SMX	0 (0)	1 (5.56)	0 (0)	1 (33.33)	2 (16.67)	0 (0)	5 (12.50)	0 (0)	9 (4.50)	0.108
CIP	10 (31.25)	0 (0)	2 (10.00)	1 (33.33)	8 (66.67)	35 (92.11)	25 (62.50)	8 (80.00)	89 (44.50)	0.04

Table 4: Antimicrobial resistance profile of the *enterococca*l isolates from the fish ponds.

Values in parenthesis represents (%); TRI: Trimethoprim; CHL: Chloramphenicol; ERY: Erythromycin; OXY: Oxytetracycline; SMX: Sulfamethoxazole; CIP: Ciprofloxacin.

Environmental water quality studies may benefit however from focusing on a subset of *Enterococcus* spp. that are associated consistently with sources of faecal pollution such as domestic sewage, rather than testing for presence of the entire genus. *E. faecium* and *E. faecalis* are potentially good focal species for such studies, as they have been consistently identified as the dominant *Enterococcus* spp. in human faeces and sewage [15]. In this study, the presence of *Enterococcus* species (*E. faecalis* and *E. faecium*) from fish ponds in Benin City, Edo State has been demonstrated. Reports from this investigation shows a high counts of *Enterococcus* species ranging from $2\pm0.00\times10^2$ to $16\pm0.13\times10^2$ cfu/ml. The findings in the study were within range when compared to the findings of [16] were 1.2×10^2 to 1.8×10^3 cfu/ml isolated from fish ponds in Kano metropolis. Enterococci which have been implicated with warm-blooded animals, their faeces, animal carcasses or milk, are also able to colonise a diversity of niches, mainly because of their exceptional aptitude and intrinsic resistance against hostile conditions [6]. Previous studies have demonstrated that enterococci have a strong relationship with swimming associated illnesses in both marine and fresh water environments [15]. The public health costs associated with exposure to faecal contamination may have a huge economic impact. A total of 104/200 (52%) *E. faecalis* and 96/200 (48%) *E. faecium* were isolated from the fish ponds in the study. Multidrug resistance profile of the isolates in the study reveals that 15 of the isolates were resistant to all antimicrobials used in the study [17]. Overuse and misuse of antimicrobials in food animals represent a public health risk as they contribute to the emergence of resistant forms of disease-causing bacteria which is a contributing factor to their pathogenesis [18].

Conclusion

The information obtained from the study reveals that *Enterococcus faecalis* and *Enterococcus faecium* were highly resistant to the action of antimicrobials used in the study, in Benin City, Edo State. These antimicrobials are usually used as prophylactics, growth promoters, and also as treatment to the fishes in the fish ponds. Resistance to these antimicrobials could be attributed to selective pressure, possession of intrinsic and acquired resistance to the antimicrobials in the fish ponds and fish pond facilities in Benin City. Adherence to adequate and proper use of manure products and frequent discharge of water from fish pond will reduce the high level of antimicrobial resistance in *Enterococcus* species isolated from fish ponds and also reduce the potential risk to human health.

References

1. Bates J, Jordens JZ, Griffith DT (1994) Farm animals as a putative reservoir for vancomycin resistant enterococcal infections in man. J Antimicrob Chemother 34(4): 507-514.

2. Bager F, Madsen M, Christensen J, Aarestrup FM (1997) Avoparcin used as a growth promoter is associated with the occurrence of vancomycinresistant *Enterococcus faecium* in Danish poultry and pig farms. Prev Vet Med 31(1-2)**:** 95-112.

3. McPhearson RM, DePaola A, Zywno SR, Motes ML, Guarino AM (1991) Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. Aquaculture 99(3-4)**:** 203-211.

4. Alderman DJ, Hastings TS (1998) Antibiotic use in aquaculture development of antibiotic resistance potential for consumer health risks. International Journal of Food Science and Technology 33(2)**:** 139-155.

5. Aarestrup FM (1995) Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from ecological and conventional poultry farms. Microb Drug Resist 1(3): 255-257.

6. Guardabassi L, Dalsgaard A, Raffatellu M, Olsen JE (2000) Increase in the prevalence of oxolinic acid resistant *Enterococcus* spp. observed in a stream receiving the effluent from a freshwater trout farm following the treatment with oxolinic acid-medicated feed. Aquaculture 188(3-4)**:** 205-218.

7. Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL (2000) Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Applied Environmental Microbiology 66(11)**:** 4908-4915.

8. McFaddin JF (1985) Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, USA.

9. Anderson NL. Cumitech 3B: Quality Systems in the Clinical Microbiology Laboratory. ASM Press, Washington DC.

10. Jensen BB (1998) The impact of feed additives on the microbial ecology of young pigs. Journal of Animal and Feed Sciences 7: 45-64.

11. Leclerq R, Derlot E, Duval J, Courvalin P (1988) Plasmid mediated resistance to vancomycin and eicoplanin in Enterococccus faecium. N Engl J Med 319: 157-161.

12. Klare I, Heier H, Claus H, Reissbrodt R, Witte W (1995) VanA-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol Lett 125(2-3): 165-172.

13. Wegener HC (1998) Historical Yearly Usage of Glycopeptides for animals and humans: the American-European paradox revisited. Antimicrob Agents Chemother 42(11): 3049.

14. CLIS (2006) Performance standards for antimicrobial susceptibility testing: Eleventh informational supplement. CLIS, Wayne, Pa.

15. Laukova A, Juris P (1997) Distribution and characterization of *Enterococcus* species in municipal sewages. Microbios 89(359)**:** 73-80.

16. McDonald CL, Kuehnert MJ, Tenover FC, Jarvis WR (1997) Vancomycin-resistant enterococci outside the health care setting: prevalence, sources, and public health. Emerg Infect Dis 3(3): 311-317.

17. Singh N, Rogers P, Atwood CW, Wagener MM, Yu VL (2000) Short-course empiric antibiotic therapy for patients with pulmonary infiltrates in the intensive care Unit: A proposed solution for indiscriminate antibiotic prescription. Am J Respir Crit Care Med 162(2): 505-511.

18. Witte W (1998) Medical consequences of antibiotic use in agriculture. Science 279(5353): 996-997.