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Occurrence of Antibiotic-resistant *Proteus mirabilis* and *Proteus vulgaris* in African Catfish (*Clarias* sp.) Isolates in Banyuwangi, East Java, Indonesia

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ABSTRACT

Background: This study seeks to evaluate the resistance profiles of *P. mirabilis* and *P. vulgaris* in isolates from African catfish in Banyuwangi, East Java, Indonesia.

Methods: A total of 74 African catfish samples were procured from 12 distinct aquaculture ponds in Banyuwangi. The catfish were intentionally selected from tarpaulin and cement ponds. The samples were categorized into two groups: G1, comprising African catfish with clinical evidence of hemorrhage and superficial ulceration and G2, consisting of those without apparent clinical symptoms. Water quality assessments were performed for each pond. Following this, *P. mirabilis* and *P. vulgaris* were isolated and identified from liver and skin samples utilizing MCA media, followed by Gram staining and biochemical assays. The identified bacterial isolates were sensitivity tested for beta-lactam and non-beta-lactam resistance.

Result: The findings indicated that *P. mirabilis* and *P. vulgaris* were present in African catfish, with prevalence rates of 4.05% (3/74) and 10.81% (8/74), respectively. *P. mirabilis* exhibited extremely high resistance rates against beta-lactams antibiotics Cefadroxil, Ampicillin, Penicillin and Oxacillin, with very high resistance to non-beta-lactam antibiotic Erythromycin (66.7%). *P. vulgaris* showed extremely high resistance to beta-lactams antibiotics Penicillin and Oxacillin and very high resistance to Cefadroxil and non-beta-lactam Erythromycin (both at 62.5%), Streptomycin (50%) and Cefixime (25%). The factor influencing the presence of *P. vulgaris* in African catfish is the type of pond ($p = 0.044$). It can be concluded that the beta-lactam antibiotics exhibit the highest and most prevalent resistance on *Proteus* spp. isolates. Additionally, resistance to other non-beta-lactam antibiotics is also observed. Greater caution should be exercised in the administration of antibiotics in aquaculture facilities to safeguard public health.

Key words: African catfish, Antibiotic resistance, Pond, Proteus spp, Public health.

INTRODUCTION

Antibiotic resistance presents a considerable challenge to public health systems, both in developing and industrialized nations. The phenomenon of antibiotic resistance is a major health concern with alarming projections indicating that related death cases could rise to 10 million annually by 2050, resulting in an economic loss nearing 100 trillion dollars due to antibacterial resistance (O'Neill, 2016; Naghavi *et al.,* 2024). Infectious diseases remain a significant health issue, particularly in developing countries. The preferred strategy for addressing this issue involves the use of antimicrobial agents, including antibiotics, antifungals, antivirals and antiprotozoals. Improper use of antibiotics may lead to the emergence and proliferation of antibiotic-resistant bacteria, highlighting the necessity for rational antibiotic use while considering the potential consequences (Thresia *et al.,* 2023). Antimicrobial resistance can adversely affect the quality of healthcare services (Hocking *et al.,* 2021).

Indonesia has issued guidelines regarding the use of antibiotics, which provide comprehensive information on the selection and application of antibiotics in specific contexts. These guidelines are anticipated to bolster public health and veterinary healthcare services, thereby contributing to the control of antimicrobial resistance in Indonesia

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(Sadikin, 2021; Nasrullah *et al.,* 2021). From a global health perspective, antimicrobial resistance is not perceived as an isolated issue; rather, it is recognized as a challenge that is interconnected with various sectors, including public health, animal health-encompassing fisheries and aquaculture-food systems and environmental health. In this context, the "One Health" approach is essential for effectively addressing antimicrobial resis-tance due to its complexity. The overuse of antibiotics and the inadequate application of standard precautions in livestock farming and veterinary healthcare are major contributors to resistance development, negatively impacting both human and animal populations and threa-tening food safety. The concurrent application of the same antimicrobial drugs in humans, livestock and aquaculture is suspected to be a critical driver in the creation and spread of resistant bacteria. The Indonesian population demonstrates a pronounced demand for catfish, with consumption levels consistently rising each year. The production of African catfish in Indonesia experienced notable growth, increasing from 841.75 thousand tons to 1.81 million tons during the period from 2017 to 2018 (The Ministry of Marine Affairs and Fisheries, 2018). In the Banyuwangi district, situated in the westernmost region of East Java, Indonesia, the output of catfish escalated from 4,470 tons in 2019 to 5,100 tons in 2020 (Department of Marine Affairs and Fisheries, 2021). African catfish aqua-culture in Banyuwangi is predominantly conducted on a small scale by local households, serving as a supple-mentary source of income for the community. Microbial infections represent a significant challenge in aquaculture, leading to annual fish mortality, job losses, economic instability and food shortages (Assefa and Abunna, 2018). Recent studies have indicated that *P. mirabilis* has emerged as a considerable threat to aquaculture (Pattanayak *et al.,* 2018; Zhai *et al.,* 2023). Research has demonstrated that various pathogens, including bacteria, viruses and parasites, can infect African catfish and induce severe illnesses. Research by Sun *et al.* (2020), indicates that *P. vulgaris* has been associated with disease outbreaks in farmed common carp (*Cyprinus carpio*) in China. Recent findings suggest that *Proteus* spp. have emerged as substantial risks to aquaculture (Mumbo *et al.,* 2023).

Proteus spp. is recognized as a human pathogen that exploits favorable conditions for proliferation. It has been observed that *Proteus* spp. can inhabit a variety of hosts, including both wild and domestic animals such as mice, mammals, birds, reptiles, amphibians and marine species, due to their symbiotic relationships within ecosystems (Drzewiecka, 2016). Specific strains of *Proteus* bacteria, notably *P. mirabilis* and *P. vulgaris*, have has been linked to urinary tract calculi, urinary tract infections and wound infections in humans (Yuan *et al.,* 2021). While *P. vulgaris* is frequently implicated in urinary tract infections (UTIs), it is less commonly isolated than *P. mirabilis*. Notably, *P. vulgaris* possesses significant motility attributed to fimbriae, as well as the ability to release various enzymes and endotoxins (Ibrahim, 2023). This study explores the resistance characteristics of *P. mirabilis* and *P. vulgaris* in African catfish, specifically those isolated from Bany-uwangi, East Java, Indonesia.

MATERIALS AND METHODS

Ethical approval

This study was approved under number 1.KEH.162.11. 2022 by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga.

Study iocation

The study was carried out from September 2022 to June 2023 at Laboratory Facilities of Faculty of Health, Medicine and Life Sciences (FIKKIA), Universitas Airlangga.

Sampling pattern

A total of 74 African catfish were divided into two groups. The first group (G1) comprised 34 individuals exhibiting clinical signs of skin hemorrhages and ulceration, while the second group (G2) consisted of individuals lacking any clinical symptoms. Participants were selected purposefully based on two distinct types of aquaculture systems, specifically tarpaulin ponds and cement ponds, belonging to various farmers. The stocking densities within these ponds ranged from 200 to 500 individuals per cubic meter. The sampled fish were promptly transported in insulated containers containing original water for subsequent laboratory analysis.

Evaluation of pond water quality

On-site measurements were conducted to assess water quality parameters, specifically monitoring the frequency of water changes, temperature fluctuations, pH levels and ammonia concentrations. Temperature, pH and ammonia levels were measured using a water thermometer (Resun, Indonesia), pH indicator paper (Merck, Germany) and a SERA ammonium/ammonia test kit (SERA, Germany), respectively.

Bacteria isolation and identification

Liver and skin lesion samples were collected using sterile cotton swabs and subsequently transferred to buffered peptone water. The swabs were cultured on MacConkey agar (HiMEDIA, USA) (37°C, 24 hours). Bacterial colonies that exhibited the characteristic morphology and coloration of non-lactose fermenters were then transferred to Eosin Methylene Blue and Blood Agar (HiMEDIA, USA) for further culturing. Following this, a gram-staining procedure was performed, noting that Proteus spp. is categorized as a gram-negative bacterium. Identification of Proteus spp. isolates was accomplished through a series of biochemical tests, which included assessments of motility, Simon citrate utilization, indole production, gas production, hydrogen sulfide production utilizing Triple Sugar Iron agar slant culture, urease activity, methyl red test and Voges-Proskauer test (Anifowose *et al.,* 2024).

Resistance test

The antibiotics (Oxoid, UK) utilized in the study included non-beta-lactams: Tetracycline (30 µg), Ciprofloxacin (5 µg), Streptomycin (10 µg), Erythromycin (15 µg) and Chloramphenicol (30 µg), along with beta-lactams: Cefadroxil (30 µg), Cefixime (5 µg), Ampicillin (10 µg) and Penicillin (10 µg). The antibiotic resistance of *P. vulgaris* and *P. mirabilis* was assessed utilizing the Kirby-Bauer disk diffusion method. A 0.1 mL bacterial suspension was inoculated to Mueller-Hinton agar (Merck, Germany). The bacterial sus-pension was uniformly spread across the agar surface using a curved glass rod to facilitate adhesion of the bacteria to the agar medium. After 10 minutes, the antibiotics was applied to the surface of the agar and the plates were incubated (37°C, 24 hours). The zones of inhibition were measured using a caliper and then contrasted to Clinical and Laboratory Standards Institute standards (CLSI, 2020).

Statistical analysis

Descriptive statistics were employed to present the data as percentages, which were organized in tables. The Chi-square statistical test that was performed using SPSS version 27.0, was utilized to identify the factors influencing the prevalence of *P. mirabilis* and *P. vulgaris*.

RESULTS AND DISCUSSION

The findings indicate that *P. mirabilis* and *P. vulgaris* were identified in African catfish at rates of 4.05% (3/74) and 10.81% (8/74), respectively. The results of the identification tests for *P. mirabilis* and *P. vulgaris* are summarized in Table 1 and Table 2, respectively. *Proteus mirabilis*, a Gram-negative rod-shaped bacterium, is well-documented for its urease production. Additionally, it is recognized for its ability to differentiate into elongated swarm cells and for exhibiting a distinctive motility pattern on agar plates that resembles a bull's-eye (Anggraini ***et al.,*** 2022). *P. mirabilis* can be isolated from a variety of habitats, including aquatic environments, soil, waste and as part of the microflora in the digestive tracts of animals and humans. This bacterium is known to cause infections, particularly in individuals with compromised immune systems and is predominantly responsible for acute urinary tract infections (Adeolu ***et al.,*** 2016; Armbruster ***et al.,*** 2018).

The prevalence of *P. mirabilis* in the sampled population was notably low, at 4.05%. Drzewiecka (2016) reported that *P. mirabilis* is part of the pathogenic or physiological microflora of animals, particularly residing in the intestines. These bacteria are present in animals, encompassing mammals, birds, reptiles, insects, amphibians, aquatic organisms also in human (Zappa ***et al.,*** 2017; Davies ***et al.,*** 2022; Ram ***et al.,*** 2022). Several preceding studies have yielded similar findings; for instance, Gufe ***et al.*** (2019) reported that only 5% of *P. mirabilis* contamination was found in fish sold in wet markets in Mufakose, Zimbabwe. Other investigations conducted in Karachi, Pakistan, indicated that only 4.14%

Table 1: Prevalence of *P. mirabilis* in the examined sample.

Group	N	Organ	Suspected Isolated stain	Gram negative	TSIA*	SIM*	Urea+	Citrat+ (slow)	MR+	VP-	Confirmed *P. mirabilis* (%)
G1	37	Hepar	7	7	1	1	1	4	6	7	1 (2.70)
		Musculus and skin	8	8	1	1	1	3	8	8	1 (2.70)
G2	37	Hepar	6	6	1	1	1	3	6	6	1 (2.70)
		Musculus and skin	8	8	0	0	3	2	8	8	0 (0.00)
Total (%)	74	Hepar and musculus and skin	29 (33.19)	29 (39.19)	3 (4.05)	3 (4.05)	6 (8.11)	12 (16.22)	28 (37.84)	29 (39.19)	3 (4.05)

**P.mirabilis* classification is TSIA reaction with a pattern of fermentation slant/botton/ H2S/Gas: AL/AC/+/+, SIM (Sulfide/Indol/ Motil) pattern of +/-/+ , sitrat +(slow), MR+ and VP-.

Table 2: Prevalence of *P. vulgaris* in the examined sample.

Group	N	Organ	Suspected Isolated (%)	Gram negative stain	TSIA*	SIM*	Urea+	Citrat-	MR+	VP-	Confirmed *P. vulgaris* (%)
G1	37	Hepar	7	7	3	3	3	3	6	7	3 (37.5)
		Musculus and skin	8	8	2	2	1	5	8	8	2 (25)
G2	37	Hepar	8	8	1	1	1	4	8	8	1 (12.5)
		Musculus and skin	9	9	2	2	3	7	9	9	2 (25)
Total (%)	74	Hepar and musculus and skin	32 (43.24)	32 (43.24)	8 (10.81)	8 (10.81)	8 (10.81)	19 (25.68)	31 (41.89)	32 (41.89)	8 (10.81)

**P.vulgaris* classification is TSIA reaction fermentation slant/botton/ H2S/Gas with a pattern of AC/AC/+/+ and SIM (Sulfide/Indol/ Motil) pattern of +/+/+, sitrat -. MR + and VP -.

of fish samples were contaminated with *P. mirabilis* (Mansoor *et al.,* 2019).

P. vulgaris, a member of the Enterobacteriaceae family, serves as a normal commensal organism within the intestinal microbiota (Kozlovska, 2023). Many strains of *P. vulgaris* can secrete the cytolytic hemolysin HpmA, which has demonstrated the ability to disrupt the integrity of blood cells, bladder epithelial cells, monocytes and B-cell lymphoma cells (Hamilton, 2018). Furthermore, Cestari *et al.* (2013) indicated that HpmA exhibits enhanced activity in lysing erythrocytes under anaerobic conditions and at different temperatures.

P. mirabilis and *P. vulgaris* demonstrated resistance to more than three beta-lactam antibiotics, as outlined in Table 3. The resistance patterns exhibited by the two Proteus species to beta-lactam drugs were highly comparable. *P. mirabilis* exhibited extremely high resistance to Cefadroxil, Ampicillin, Penicillin and Oxacillin; very high resistance to Erythromycin (66.7%); and high resistance to Cefixime (33.3%). In contrast, other non-beta-lactam antibiotics, including Tetracycline, Ciprofloxacin, Streptomycin and Chloramphenicol, demonstrated sensitivity. Table 3 further demonstrates that *P. vulgaris* has profound resistance to Penicillin and Oxacillin, alongside notably high resistance to Cefadroxil and Erythromycin, at 62.5% each. Furthermore, it demonstrates substantial resistance to the antibiotics Streptomycin and Cefixime. In contrast to the findings of Ronanki *et al.* (2022), which demonstrated that *P. mirabilis* and *P. vulgaris* isolated from milk and meat samples in Tirupati, Andhra Pradesh, showed complete resistance (100%) to Tetracycline, Colistin, Erythromycin and Penicillin-G; also the study by Talebi *et al.* (2023) on *P. mirabilis* and *P. vulgaris* from clinical samples in Iran revealed 100% resistance to penicillin and tetracycline. This study suggests that *P. mirabilis* and *P. vulgaris* are still susceptible to Tetracycline.

The two Proteus species, display differences in their susceptibility to non-beta-lactam antibiotics. *P. mirabilis* exhibited resistance to the non-beta-lactam antibiotic Erythromycin, whereas *P. vulgaris* demonstrated resistance to the non-beta-lactam medication Streptomycin. This discrepancy may be attributed to the isolation of the two bacterial species from African catfish residing in distinct types of aquaculture ponds. The presence of *Proteus* spp. in African catfish is influenced by multiple factors, as evidenced by the results of the factor analysis. Table 4 provides an overview of a number of characteristics, such as the kind of pond, the age of the African catfish and the existence of clinical indications like ulcerations and hemorrhages on the skin. Additionally, it includes data regarding water quality and frequency. These variables did not appear to influence the presence of *P. mirabilis* in the liver, mucosa and skin of the fish. However, the type of pond emerged as a significant determinant ($p = 0.044$) to the presence of *P. vulgaris* in African catfish.

A total of twelve ponds, comprising five tarpaulin ponds and seven cement ponds, yielded 74 catfish samples. According to The National Standardization Agency of Indonesia (2014), African catfish should be cultivated in water that maintains a temperature of 25-30°C, a pH of 6.5-8 and a maximum ammonia concentration of 0.1 mg/L. The water quality in the study's three catfish cultivation ponds exhibited pH and ammonia levels that were outside the established ranges. However, as noted in Table 4, these water quality variables did not significantly impact the presence of *P. vulgaris* or *P. mirabilis* isolated from samples of African catfish.

Continuous vigilance is imperative to mitigate the dissemination of antibiotic resistance in *P. mirabilis* and *P. vulgaris* found in African catfish consumed by the community. While certain resistance mechanisms may develop without progressing beyond initial stages, others possess the potential for rapid global dissemination, akin to a chain reaction (Zhang and Cheng, 2022). This phenomenon arises from the capability of microorganisms to acquire resistance to the antimicrobial agents employed against them. Furthermore, many pathogenic organisms can develop resistance to at least some antimicrobial agents through various mechanisms, including changes of target sites, reduced influx, enhanced efflux pumps and enzymatic

Table 3: Antimicrobial resistance profile of *P. mirabilis* and *P. vulgaris* isolates.

Type of antibiotic	Antimicrobials (µg)	*P. mirabilis* (n=3)	*P. vulgaris* (n=8)
		R	R
Non beta lactams	Tetracycline (TE 30 µg)	0 (0.0)	0 (0.0)
	Ciprofloxacin (CIP 5µg)	0 (0.0)	0 (0.0)
	Streptomycin (S 10µg)	0 (0.0)	4(50.0)*
	Erythromycin (E 15µg)	2 (66.7)**	5(62.5)**
	Chloramphenicol (C 30 µg)	0 (0.0)	0 (0.0)
Beta lactams	Cefadroxil (CFR 30 µg)	3 (100.0)***	5(62.5)**
	Cefixime (CFM 5µg)	1 (33.3)*	2(25.0)*
	Ampicilin (AMP 10µg)	3 (100.0)***	5(62.5)**
	Penicilin (P 10µg)	3 (100.0)***	6(75.0)***
	Oxacilin (OX 1µg)	3 (100.0)***	8(100.0)***

Low: ≤10% Moderat: >10% to 20%; *High: >20% to 50%; **Very High: >50% to 70%,*** Extremely high: >70%.

Table 4: Factors influencing the presence of *P. mirabilis* and *P. vulgaris* in African catfish.

Variable	*P. mirabilis*		*P. vulgaris*	
	Total	p-value	Total	p-value
Types of ponds		0.908		0.044*
Cement pond (n=47)	2		2	
Tarpaulin pond (n=27)	1		5	
The age of African catfish can		0.180		0.200
<4 months (n=47)	3		6	
e”4 months (n=27)	0		1	
African catfish condition		0.556		0.691
Normal, without any clinical symptoms (n=37)	1		3	
Clinical signs of skin haemorrhages, ulceration (n=37)	2		4	
Frequency of water replacement		0.588		0.264
Weekly (n=36)	1		2	
Biweekly or less frequently (n=38)	2		5	
pH water		0.123		0.428
<6.5 (n=36)	0		2	
6.5-8.0 (n=38)	3		5	
Amoniac value		0.757		0.468
e”0.1 mg/L (n=55)	2		6	
<0.1 mg/L (n=19)	1		1	

* indicates a significant difference (p<0.05).

inactivation of antibacterials (Belay *et al.,* 2024). The inherent capacity of microorganisms to acquire resistance poses a risk to both animal and human health, while also resulting in the accumulation of antibiotic residues in animal products (Shukla *et al.,* 2022).

CONCLUSION

The discovery of *P. mirabilis* and *P. vulgaris* in African catfish represents a significant public health concern, as these bacteria have the potential to cause illness in humans. The identification of microbial resistance is critical, with potentially far-reaching implications for public health. Studies on *Proteus* spp. isolates have indicated that beta-lactam antibiotics exhibit the highest levels of resistance. Moreover, resistance is also observed to other classes of antibiotics that are not beta-lactam. Hence, caution is warranted in the administration of antibiotics within aquaculture practices to safeguard public health.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved under number 1.KEH.162.11.2022 by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga.

Author contributions

All authors have contributed equally to this work.

Conflict of interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript.

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