Preliminary Study of Incidence of MDR *Burkholderia cepacia* in Nile Tilapia in Egypt

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Abstract

It is not well known if Burkholderia cepacia can be found in fish or water. This is because bacteria may enter into the water through sewage, animal waste, and soil runoff. Therefore, the current study was carried out to assay the incidence of biochemically identified *Burkholderia cepacia* in seventy-five Nile Tilapia and twenty-five water samples collected from Elmanzala Lake, Egypt, with API 20 NE and its antibiotic resistance. Three *B.cepacia* isolates were identified from 100 samples with aprevalence 3%. Two from fish (only gills) with a prevalence 2.7%(2/75) and one from the water sample with a prevalence 4%(1/25). Only one isolate was MDR with 0.43 MAR index. Besides, all isolates were susceptible to levofloxacin, meropenem, and chloramphenicol, while, recovered isolates were resistant to ceftazidime (100%), penicillin G(66.6%), and trimethoprim/ sulfamethoxazole (33.4%). This study concluded that MDR *B.cepacia* is of importance in public health, and is considered human health issue.Meropenem ,cholramphenicol and levofloxacin used as drug of choice for treatment *B.cepacia.*

Keywords: *Burkholderia cepacian,*Nile Tilapia,MDR

1. Introduction

The most significant disease concerns in aquaculture are caused by bacterial infections, which are naturally present in fish environments**.** Burkholderia are non-spore-forming, Gram-negative aerobic bacteria. These organisms are adaptable to several biological environments, including soil, water, animals, and human respiratory tracts ***(Coenye and Vandamme, 2003)***.

Burkholderia, a multi-species bacterial genus, has nine phenotypically similar species known as the *Burkholderia cepacia* complex (Bcc) ***(Luvizotto and Marcon, 2010). (Yabuuchi et al. 1992)*** named the genus Burkholderia after Burkholder, who discovered it but classified it as *Pseudomonas* ***(Burkholder, 1950).***

B. cepacia complex(Bcc) as a food-spoilage organism. Bcc is a life-threatening infection, especially in individuals with cystic *fibrosis* ***(LiPuma, 1998a)*** and chronic granulomatous disease ***(Speert et al., 1994)***. Some cases of endocarditis and nosocomial infection outbreaks have been associated with *B. cepacia* ***(Hirose et al., 1998; Kaitwatcharachai et al., 2000).*** Although reports of Bcc-caused animal illnesses recorded ***(Berriatua et al., 2001)***, the distribution of Bcc in animal species and its transmission are generally not well reported.

The extensive usage of antimicrobial drugs has assisted in the emergence of multidrug-resistant organisms ***(Levy, 2002,Algammal et al., 2022a)***. Most antimicrobial drugs do not affect Bcc, Effective treatments are not always easy to understand Thus, the primary objective of management efforts is the prevention of related infections ***(LiPuma, 1998b,Algammal et al., 2022b)***. Thus, the purpose of this investigation is to identify the prevalence of *B. cepacia* in fish and to evaluate its possible role as a source of infection especially from water with its antibiotic resistance.

2. Materials and methods

2.1. Sampling

Seventy-five diseased Nile tilapia (*Oreochromis niloticus*) showed signs of petechial haemorrhages anderosions on skin and twenty-five water samples with normal paremeters were obtained from Elmanzala Lake, Egypt from june to september 2023. Further clinical and bacteriological assessments of the collected fish were carried out in aerated sealed plastic bags in the laboratory.

2.2.Isolation of *Burkholderia cepacia*

Loopfuls from the fish(liver, kidney, gills), and water samples were examined to assess the presence of *Burkholderia cepacia*, which was isolated and identified ***(Henry et al., 1997)***. The samples were inoculated on trypticase soya agar (Oxoid, UK) with 5% sheep blood(Oxoid, UK) and macCkongy agar (Oxoid, UK), and the plates were incubated at 35 °C for 24 hours.

2.3. *Burkholderia cepacia* Identification

Microscopical examination of Gram-stained films and culture characterization, All cultures that produce a positive oxidase reaction were classified as prospective *Burkholderia cepacia* and were kept for further identification using the API 20 NE system (bioMérieux, SA, France). Catalase, nitrate reduction, gelatin hydrolysis, Lysine decarboxylase, and Ornithine decarboxylase, indole, glucose fermentation, arginine hydrolysis, and urease were performed on suspected colonies.

2.4.Antibiogram Testing

Antimicrobial sensitivity of *Burkholderia cepacia* isolates

(NCCLS standards (1997) by disk diffusion method against seven antimicrobial agents categorized into seven classes including penicillin G(PEN) (10 U), amoxicillin-clavulanic acid (AMC) (30 µg), meropenem (MEM) (10 µg), ceftazidime (CAZ) (30 µg),levofloxacin (LEV) (5 µg), sulfamethoxazole/trimethoprim (SXT) (25 µg), and chloramphenicol (C) (25 µg).

3. Results

3.1. Prevalence of *Burkholderia cepacia* among Examined Fish

Morphologically, all the retrieved *Burkholderia cepacian*(*B.cepacia*) isolates were Gram-negative, bacilli. The colonies on macCkongy agar plates were pale and non-lactose fermenter (Figure 1A) and gave beta hemolysis on blood agar (Figure 1B). Biochemically, all the recovered isolates were positive for oxidase, catalase, nitrate reduction, gelatin hydrolysis, Lysine decarboxylase, and Ornithine decarboxylase. While all the recovered isolates were negative for indole, glucose fermentation, arginine hydrolysis, and urease (Table 1). In addition, the API20NE confirmed the identification of all obtained isolates with a probability of 100%(454557). The prevalence of *Burkholderia cepacia* among the examined samples was 3% (3/100). Two from fish (only gills) with a prevalence 2.7%(2/75) and one from the water sample with a prevalence 4%(1/25).

3.2. Antibiogram results

The result of the antibiogram of three strains isolated from Nile tilapia. Table (2) revealed that three isolates were resistant to ceftazidime(100%), penicillin G(66.6%), and trimethoprim/ sulfamethoxazole (33.4%) and sensitive to levofloxacin, meropenem, and chloramphenicol (100%) and one isolate considered as MDR. MARI was determined as 0.14 to 0.43.

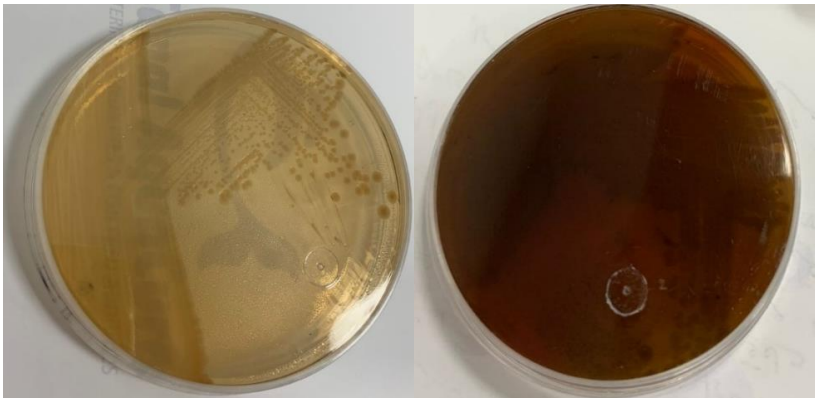

Figure 1 A: Pale and non-lactose colonies on macCkongy agar. **B**:Beta hemolysis colonies on blood agar.

Table 1 : Biochemical identification of *B.cepacia*

Biochemical test	***B.cepacia***
Oxidase	Positive
Catalase	Positive
Nitrate reduction	Positive
Urease	Negative
Indole	Negative
Glucose fermentation	Negative
Gelatin hydrolysis	Positive
Lysine decarboxylase	Positive
Ornithine decarboxylase	Positive
arginine hydrolysis	Negative

Table 2:Antibiogram results of recovered isolates

Antibiotic classes	Antibiotic agent	Sensitive	Resistant
Penicillins	Penicillin G	1(33.4%)	2(66.6%)
	Amoxacillin/Clavulanic acid	3(100%)	0(0)
Cephalosporin	Ceftazidime	0(0)	3(100%)
Carpebenem	Meropenem	3(100%)	0(0)
Fluoroquinolone	Levofloxacin	3(100%)	0(0)
Phenicols	Chloramphenicol	3(100%)	0(0)
Sulfonamides	Trimethoprim/ sulfamethoxazole	2(66.6%)	1(33.4%)

Discussion

Burkholderia cepacia in water and fish indicated high contamination. In addition, this study aimed to detect *Burkholderia cepacia* in fish and its antibiotic sensitivity. Our study revealed an incidence of *B.cepacia* 2.7%(2/75) in Nile Tilapia and 4% in the water samples with its total prevalence (3%) similar to ***(El-Barbary and Hal ,2017)***. who identified three *B.cepacia* isolates in fish. Bcc in food is a major problem because of its capacity to proliferate at low temperatures and its emergence as an opportunistic pathogen, particularly for cystic fibrosis patients, which can cause life-threatening infections ***(Nagah et al., 2012)***.

Several studies have shown problems with phenotypic approaches misidentifying Burkholderia species ***(Kiska et al., 1996, Henry et al., 1997 Henry et al., 2001).***Phenotypic classification approaches may not be sufficient for identifying *Burkholderia cepacia*, although the API 20NE successfully identified all three strains recovered from fish.

(Wiedmann et al., 2000) found that API 20NE accurately identified *Pseudomonas* isolates and ***(El-Barbary and Hal, 2017)*** identified *Burkholderia cepacia.*

These findings support the occurrence of MDR *B.cepacia* isolates in aquaculture sectors and demonstrate that the food chain might spread MDR pathogens between animals and humans (Algammal et al., 2022a). Randomly treating developing infections with antibiotics may cause MDR strains in aquaculture. Thus, routine antibiotic susceptibility testing is essential for selecting effective antibiotics and resolving such issues(Algammal et al., 2022b). Upon examining *B.cepacia* antibiotic sensitivity in Table 2, isolates showed resistance to multiple antibiotics. All isolates proved resistant to ceftazidime (100%), while 33.4% were susceptible to penicillin and 66.6% sensitive to Trimethoprim/ sulfamethoxazole. ***(Isles et al., 1984)*** discovered that Bcc isolates were resistant to ampicillin (97%), gentamicin (97%), and chloramphenicol (45%). Additional studies on Bcc species revealed extensive resistance ***(Nzula et al., 2002; Zhou et al., 2007)***.

Conclusion

This study found MDR *Burkholderia cepacia* in Nile Tilapia and water which can cause infection as first reported in Egypt.Meropenem ,cholramphenicol and levofloxacin used as drug of choice for treatment *B.cepacia*. *Burkholderia cepacia* is antibiotic-resistant, and people infected may not respond to treatment. *Burkholderia cepacia* eradication as a human pathogen will become more emergent.

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الملخص العربي

دراسة أولية لحدوث عقار بيركهولديريا سيباسيا المقاوم للأدوية المتعددة في البلطي النيلي في مصر

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لم يتم توثيق وجود بكتيريا *Burkholderia cepacia* بشكل جيد في الأسماك والمياه حيث يمكن للبكتيريا أن تدخل الماء من خلال التربة ومياه الصرف الصحي وفضلات الحيوانات. لذلك، تم إجراء الدراسة الحالية لتقدير حدوث *Burkholderia cepacia* في خمسة وسبعين عينه من أسماك البلطي النيلي وخمسة وعشرين عينة مياه تم جمعها من بحيرة المنزلة، مصر، عن طريق العزل والتعرف الكيميائي الحيوي باستخدام API 20 NE والمقاومه للمضادات الحيوية. تم تشخيص ثلاث عزلات من بكتيريا *B.cepacia* من 100 عينة. عزلتان من الأسماك (الخياشيم فقط) بنسبة انتشار 2.7% (75/2) وعزله من عينة المياه بنسبة انتشار 4% (25/1). عزلة واحدة فقط كانت MDR بمؤشر MAR 0.43. الى جانب ذلك، كانت جميع العزلات حساسة لليفوفلوكساسين، الميروبينيم، والكلورامفينيكول، في حين كانت العزلات المستردة مقاومة للسيفتازيديم (100٪)، البنسلين ج (66.6٪)، وتريميثوبريم / سلفاميثوكسازول (33.4٪). وتم الاستنتاج في هذه الدراسة إلى أن MDR *B.cepacia* له أهمية للصحة العامة، ويعتبر قضية صحية تهدد لإنسان.