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Prevalence and antibiotic resistance patterns of Aeromonas and Pseudomonas species recovered from aquatic foods sold at the retail market in Egypt.

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ABSTRACT

Overuse of antibiotics in the fish and shellfish production systems throughout time may be one of the primary causes of growing antibiotic resistance in bacteria. In this study, 90 samples of Nile Tilapia, Mugil, and shrimps were tested for the presence of Aeromonas and Pseudomonas species and monitored their antibiotic resistance pattern. The isolated bacterial strains were identified based on morphological and biochemical characteristics. Further, the isolates were tested against 14 antimicrobial agents using the disc diffusion method, and the multidrug resistance pattern was studied. The results showed that across the three sample types, *Pseudomonas fluorescens* (26.7%) and *Aeromonas hydrophila* (21.1%) were the most common, followed by *A. caviae* (14.4%), *P. putrefaciens* (13.3%), *A. sobria* (12.2%), *P. fragi* (11.1%), *P. alcaligenes* (10%), *A. veronii* (5.6%), *P. proteolytica* (4.4%), *P. aeruginosa* (3.3%), *P. cepacian* (2.2%), and *A. fluvialis* (1.1%). The two most common strains were submitted for susceptibility analysis that revealed multidrug-resistant. For *Aeromonas hydrophila* the highest rates of resistance were to streptomycin (100%) and penicillin G (92.3%), then erythromycin (84.6%) and cefotaxime (84.6%). For *Pseudomonas fluorescens* highest rates of resistance were to Nalidixic acid (100%) and streptomycin (100%), then erythromycin (91.7%), penicillin G (79.2%) and cephalothin (75%). *Pseudomonas fluorescens* and *Aeromonas hydrophila* had average multiple antibiotic resistance indexes of 0.521 and 0.494, respectively. In conclusion, fish, and shellfish sold in the Egyptian market act as a reservoir for the multi-resistant Aeromonas and Pseudomonas genera. These significant findings call for effective risk assessment models and management plans that protect human, and animal.

1. INTRODUCTION

Aquatic foods are a good source of dietary protein with high nutritional values and are highly digestible, making them suitable for infants, children, and the elderly (Gufe et al., 2019). Aquatic foods contribute around 17% of animal proteins and 7% of total proteins globally. Aquatic foods offer at least 20% of the average per capita animal protein consumption for 3.3 billion people (FAO, 2022). Fish and shellfish are a potential health risk since they harbor important human pathogenic bacteria on or inside them, causing illness through improper handling and the consumption of contaminated fish and shellfish (Dissasa et al., 2022).

Aeromonas and *Pseudomonas* species are common in the aquatic environment and processing equipment, and most of them are zoonotically linked to serious human diseases (Ayoub et al., 2021). Among *Aeromonas* sp., *Aeromonas hydrophila* is one of the most known human infections (Ibraheem et al., 2017). Many *Pseudomonas* species are pathogenic for humans and aquatic organisms; *Pseudomonas fluorescens* is among the main causes of septicemic diseases among fish and shellfish, causing severe economic losses and human diseases (Circella et al., 2020). The rise of AMR has resulted in ineffective conventional antibiotics and a growing failure rate for infection treatment, leading to increased mortality rates for common infectious illnesses (Sabeq et al., 2022). Several studies (Sonkol et al., 2020; Zdanowicz et al., 2020; Thomassen et al., 2022) suggested that *Aeromonas* and *Pseudomonas* species how multiple antibiotic resistance (MDR). Antibiotic resistance can be passed through improper handling and consuming contaminated fish and shellfish (Ferri et al. 2022). Aquatic foods sold in markets carry pathogenic bacteria, and the fast growth of MDR bacteria in the food chain is concerning and poses a major hazard to the population.

Monitoring antibiotic resistance in indicators and zoonotic bacteria can provide such information. Therefore, this study aimed to determine the prevalence and investigate antibiotic resistance profiles among *Aeromonas* and *Pseudomonas* strains isolated from fish and shellfish distributed in the Egyptian Market.

2. MATERIAL AND METHODS

2.1. Samples collection and preparation

A total of ninety samples of Nile Tilapia (*Oreochromis niloticus*), Grey Mullet (*Mugil cephalus*), and Shrimp (*Metapenaeus monoceros*), with thirty samples for each. These fish were collected from local markets at Al Qalyubia Governorate and transported to the laboratory in sterile plastic bags in ice boxes within one hour for microbiological

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analysis. Sample preparation is performed according to ISO 6887-3: 2017 (ISO, 2017).

2.2. Isolation and identification

A 25 g of each aquatic food sample was weighed and homogenized in a sterile stomacher bag with 225 ml of buffered peptone water (Oxoid, UK) for 2 minutes at 320 rpm followed by overnight incubation at 37°C. From this broth, 100 µL was directly spread on selective agar plates. *Pseudomonas* species were isolated on Cephalothin-sodium fusidate-cetrimide (CFC) agar plates (HiMedia, India) and incubated at 25 °C for 48 hours (ISO, 2010). *Aeromonas* species were isolated on Aeromonas Medium Base (Ryan's medium) (Oxoid, UK) and incubated at 30°C for 48 hours (Scarano et al., 2018). Suspect colonies with typical *Aeromonas* and *Pseudomonas* morphology were biochemically confirmed using the API 20NE® system (BioMerieux, France).

2.3. Antimicrobial susceptibility testing

The identified *Aeromonas hydrophila* and *Pseudomonas fluorescens* isolates were sub-cultured twice on tryptic soy agar plates at 37 °C for 20 hrs to prepare a bacterial suspension. The antimicrobial susceptibility tests were then performed using the disk diffusion method. Four to five isolated colonies from each *Aeromonas hydrophila* and *Pseudomonas fluorescens* identified isolates were selected from a pure culture plate. All results were interpreted following the Clinical and Laboratory Standards Institute (CLSI, 2017). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were used in antimicrobial susceptibility determination as quality control organisms. All identified *Aeromonas hydrophila* and *Pseudomonas fluorescens* isolates were initially tested for resistance to fourteen (14) widely available therapies in the Egyptian fish production sector, including amikacin (AK,10 µg), ampicillin (AMP, 25 µg), cefotaxime (CTX,5 µg), cephalothin (CEP, 30 µg), ciprofloxacin (CIP,5 µg), kanamycin (K,1000 µg), gentamicin (GEN, 10 µg), nalidixic Acid (NA, 30 µg), tetracycline (TE, 30 µg), streptomycin (HLS,300 µg), penicillin-G (P, 2 units), doxycycline (DO, 10 µg), sulfamethoxazole (SXT, 25 µg), erythromycin (E, 15 µg) (HiMedia India Pvt. Ltd., Bengaluru, India). The isolates that exhibited resistance to at least three classes of antimicrobial agents tested were considered multidrug-resistant (MDR).

2.4. The multiple antibiotic resistance index

The multiple antibiotic resistance index (MARI) was computed by dividing the total number of antibiotics used in the study by the number of antibiotics to which the bacterial isolate was resistant (Krumperman, 1983) using the following formula: MARI =X/Y

Where 'X' is the number of antimicrobial agents to which bacteria revealed resistance, while 'Y' is the total number of antimicrobial agents tested. The MAR index was calculated for all the *Aeromonas hydrophila* and *Pseudomonas fluorescens* isolates.

2.5. Statistical Analysis

Statistical analysis was performed using STATA 17 (STATA Inc., USA) using descriptive statistics such as frequency, percentage, and proportion. To visualize the *Pseudomonas* and *Aeromonas* strains' multidrug resistance profile, UpSetR plot was prepared using an online platform (Lex et al., 2014).

3. RESULTS

In the present study, the abundance of *Aeromonas* and *Pseudomonas* species in three aquatic foods, mainly Nile Tilapia (*Oreochromis niloticus*), Grey Mullet (*Mugil Cephalus*), and shrimp (*Metapenaeus monoceros*), were determined. The data on the abundance of bacteria representing the genus *Aeromonas* and *Pseudomonas* in three different aquatic food types yielded 94 isolates of interest (Table 1). According to these data, Shrimp samples contained large numbers of *Pseudomonas* (76.67%) *(23/30)* and *Aeromonas* (50%) (15/30) species, followed by Nile tilapia *Pseudomonas* (63.3%) *(19/30)* and *Aeromonas* (26.67%) (8/30) then Grey Mullet contains *Pseudomonas* (60%) *(18/30)* and *Aeromonas* (23.3%) (7/30). The predominant species were *Pseudomonas fluorescens* (26.67%) (24/90) then *Aeromonas hydrophila* (14.44%) (13/90) (Figure 1).

Table 1 Incidence of *Aeromonas* and *Pseudomonas* species in the examined aquatic food samples (n=30).

Strains	*Food Samples*		
	Nile Tilapia	*Grey Mullet*	*Shrimps*
Aeromonas sobria	10% (3/30)	13.33% (4/30)	13.33% (4/30)
Aeromonas hydrophila	13.33% (4/30)	6.67% (2/30)	23.33% (7/30)
Aeromonas veronii	3.33% (1/30)	3.33% (1/30)	10% (3/30)
Aeromonas fluvialis	___	___	3.33% (1/30)
Pseudomonas alcaligenes	16.67% (5/30)	10% (3/30)	3.33% (1/30)
Pseudomonas fluorescens	23.33% (7/30)	20% (6/30)	36.67% (11/30)
Pseudomonas fragi	10% (3/30)	13.33% (4/30)	10% (3/30)
Pseudomonas putrefaciens	10% (3/30)	13.33% (4/30)	16.67% (5/30)
Pseudomonas proteolytica	___	3.33% (1/30)	10% (3/30)
Pseudomonas aeruginosa	3.33% (1/30)	___	6.67% (2/30)
Pseudomonas cepacia	___	___	6.67% (2/30)

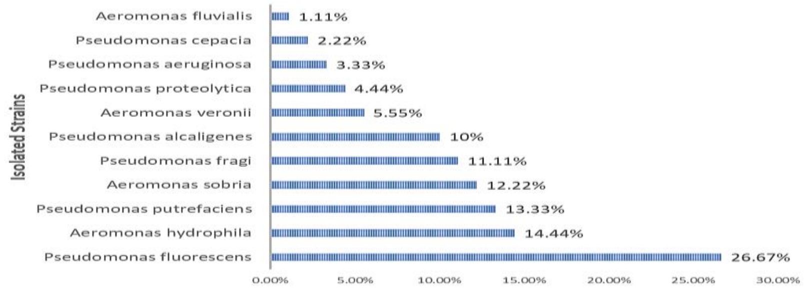

Figure 1 Prevalence rate of the isolated *Aeromonas* and *Pseudomonas* bacteria from aquatic food samples

A total of 14 antibiotics belonging to seven antimicrobial classes were used in the analysis. The antibiotic sensitivity test of the 13 strains of *Aeromonas hydrophila* and 24 strains of *Pseudomonas fluorescens* revealed these isolates' relatively high antibiotic resistance properties. The *Aeromonas hydrophila* isolates showing resistance (Table 2) to the Aminoglycoside class of antibiotics were as follows: streptomycin (100%), amikacin (30.8%), kanamycin (23.1%) and gentamicin (15%). On the other hand, resistance to the *β-lactams* class of antibiotics was as follows: penicillin G (92.3%), cefotaxime (84.6%), cephalothin (61.5%), ampicillin (7.7%). Moreover, resistance to erythromycin belongs to macrolides (84.6%), nalidixic acid (53.8%) belongs to quinolone, and sulfamethoxazole (53.8%) belongs to sulfonamides was also recorded. However, high sensitivity of the *Aeromonas hydrophila* isolates was detected to ciprofloxacin (84.6%), doxycycline (61.5), and tetracycline (53.8%).

The MDR profiles of the *Aeromonas hydrophila* isolates are presented in Figure 2. Of note, one of the *Aeromonas hydrophila* isolates showed resistance to all 14 antibiotics belonging to seven antimicrobial classes. In addition, MDR to 9–12 antibiotics belonging to six antimicrobial classes were observed in five *Aeromonas hydrophila* isolates. Other isolates showed resistance to 7 antibiotics belonging to five antimicrobial classes. Four *Aeromonas hydrophila* isolates showed resistance to 4–5 antibiotics belonging to three antimicrobial classes, and MDR to two and one antibiotic classes was found in one isolate for each.

Table 2 Antimicrobial susceptibility of *Aeromonas hydrophila* (n= 13) isolates recovered from aquatic foods.

Antibiotics	Antimicrobial Classes	Antimicrobial susceptibility					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Amikacin (AK)	Aminoglycoside	7	53.8	2	15.4	4	30.8
Ampicillin (AMP)	β-lactams	12	92.3	-	-	1	7.7
Cefotaxime (CTX)	β-lactams	-	-	2	15.4	11	84.6
Cephalothin (CEP)	β-lactams	2	15.4	3	23.1	8	61.5
Ciprofloxacin (CIP)	Fluoroquinolone	11	84.6	1	7.7	1	7.7
Doxycycline (DO)	Tetracyclines	8	61.5	-	-	5	38.5
Erythromycin (E)	Macrolides	1	7.7	1	7.7	11	84.6
Gentamicin (GEN)	Aminoglycoside	10	76.9	1	7.7	2	15.4
Kanamycin (K)	Aminoglycoside	10	76.9	-	-	3	23.1
Nalidixic acid (NA)	Quinolone	4	30.8	2	15.4	7	53.8
Penicillin G (P)	β-lactams	-	-	1	7.7	12	92.3
Streptomycin (HLS)	Aminoglycoside	-	-	-	-	13	100
Sulfamethoxazole (SXT)	Sulfonamides	5	38.5	1	7.7	7	53.8
Tetracycline (TE)	Tetracyclines	7	53.8	1	7.7	5	38.5

% Estimated according to the number of tested isolates for antibiotic sensitivity test (n = 13).

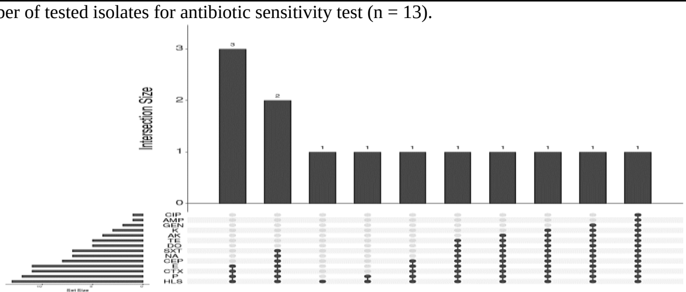

Figure 2 An UpSetR plot shows a multidrug-resistant profile among the 13 *Aeromonas hydrophila* strains detected in aquatic food samples.

The *Pseudomonas fluorescens* isolated showed resistance to Quinolone, Aminoglycoside, Macrolides, and β-lactams antibiotic classes (Table 3). All *Pseudomonas fluorescens* isolates were resistant to nalidixic acid (100%) and streptomycin (100%). High levels of resistance towards erythromycin (91.7%), penicillin G (79.2%), and cephalothin (75%) were also detected. For *Pseudomonas fluorescens,* the MDR profiles are shown in Figure 3. Three of the 24 *Pseudomonas fluorescens* isolates showed resistance to 13-14 antibiotics belonging to seven antimicrobial classes, and five isolated showed resistance to 10-12 antibiotics belonging to six antimicrobial classes. MDR to 8–9 antibiotics belonging to five antimicrobial classes were observed in five *Pseudomonas fluorescens* isolates. Eight *Pseudomonas fluorescens* isolates showed resistance to 4–6 antibiotics belonging to four antimicrobial classes. In addition, MDR to three and two antibiotic classes was found in three and two isolates, respectively.

Table 3 Antimicrobial susceptibility of *Pseudomonas fluorescens* (n=24) isolates recovered from aquatic foods.

Antibiotics	Antimicrobial Classes	Antimicrobial susceptibility					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
Amikacin (AK)	Aminoglycoside	13	54.2	2	8.3	9	37.5
Ampicillin (AMP)	β-lactams	16	66.7	1	4.2	7	29.2
Cefotaxime (CTX)	β-lactams	12	50.0	1	4.2	11	45.8
Cephalothin (CEP)	β-lactams	3	12.5	3	12.5	18	75.0
Ciprofloxacin (CIP)	Fluoroquinolone	21	87.5	-	-	3	12.5
Doxycycline (DO)	Tetracyclines	15	62.5	1	4.2	8	33.3
Erythromycin (E)	Macrolides	-	-	2	8.3	22	91.7
Gentamicin (GEN)	Aminoglycoside	22	91.7	1	4.2	1	4.2
Kanamycin (K)	Aminoglycoside	7	29.2	3	12.5	14	58.3
Nalidixic acid (NA)	Quinolone	-	-	-	-	24	100
Penicillin G (P)	β-lactams	1	4.2	4	16.7	19	79.2
Streptomycin (HLS)	Aminoglycoside	-	-	-	-	24	100
Sulfamethoxazole (SXT)	Sulfonamides	9	37.5	4	16.7	11	45.8
Tetracycline (TE)	Tetracyclines	17	70.8	3	12.5	4	16.7

% Estimated according to the number of tested isolates for antibiotic sensitivity test (n = 24).

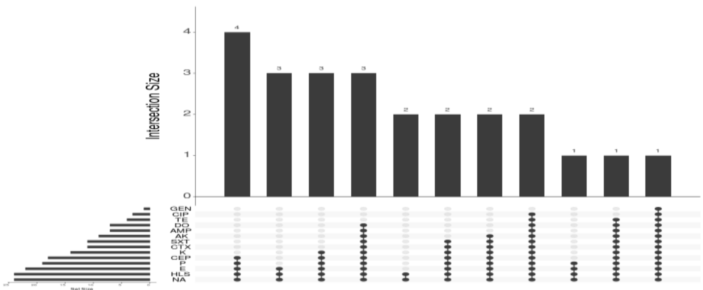

Figure 3 An UpSetR plot shows a multidrug-resistant profile among the 24 *Pseudomonas fluorescens* strains detected in aquatic food samples.

The MAR index values ranged from 0.071 to 1, with an average of 0.494 for *Aeromonas hydrophila* strains categorized under high-risk contamination. The MAR index of all *Aeromonas hydrophila* isolates shows values higher than 0.2, except for three isolates with a MAR index of 0.2 or less (Table 4). The MAR index values for the *Pseudomonas fluorescens* strains ranged from 0.143 to 1, averaging 0.512. All *Pseudomonas fluorescens* isolates show values higher than 0.2, except three with a MAR index of 0.2 or less (Table 4).

Table 4 Antibiotic resistance phenotypes and multiple antibiotic resistance (MAR) index of *Aeromonas hydrophila* strains (n=13) and *Pseudomonas fluorescens* strains (n=24) isolated from aquatic foods.

Resistance pattern	Resistance profile	Number of isolates	Number of antibiotics	MAR index
Aeromonas hydrophila				
I	HLS, P, CTX, E, CEP, NA, SXT., TE, DO, AK, K, GEN, CIP, AMP	1	14	1
II	HLS, P, CTX, E, CEP, NA, SXT, TE, DO, AK, K, GEN	1	12	0.857
III	HLA, P, CTX, E, CEP, NA, SXT, TE, DO, AK, K	1	11	0.786
IV	HLA, P, CTX, E, CEP, NA, SXT, TE, DO, AK	1	10	0.714
V	HLA, P, CTX, E, CEP, NA, SXT, TE, DO	2	9	0.643
VI	HLA, P, CTX, E, CEP, NA, SXT	1	7	0.500
VII	HLA, P, CTX, E, CEP	1	5	0.357
VIII	HLA, P, CTX, E	3	4	0.286
IX	HLS, P	1	2	0.143
X	HLS	1	1	0.071
	Average MAR index		0.494	
Pseudomonas fluorescens				
I	HLS, P, CTX, E, CEP, NA, SXT., TE, DO, AK, K, GEN, CIP, AMP	1	14	1
II	NA, HLS, E, P, CTX, K, SXT, CEP, AK, DO, AMP, TE, CIP	2	13	0.928
III	NA, HLS, E, P, CTX, K, SXT, CEP, AK, DO, AMP, TE	1	12	0.857
IV	NA, HLS, E, P, CTX, K, SXT, CEP, AK, DO, AMP	3	11	0.786
V	NA, HLS, E, P, CTX, K, SXT, CEP, AK, DO	1	10	0.714
VI	NA, HLS, E, P, CTX, K, SXT, CEP, AK	1	9	0.643
VII	NA, HLS, E, P, CTX, K, SXT, CEP	2	8	0.571
VIII	NA, HLS, E, P, CTX, K	3	6	0.428
IX	NA, HLS, E, P, CTX	4	5	0.357
X	NA, HLS, E, P	1	4	0.286
XI	NA, HLS, E	3	3	0.214
XII	NA, HLS	2	2	0.143
	Average MAR index		0.521	

4. DISCUSSION

Aeromonas and *Pseudomonas* isolated from fish and shellfish were reported earlier (Gufe et al., 2019; Morshdy et al., 2022; Thomassen et al., 2022). *P. fluorescens* and *A. hydrophila* representing widespread bacterium identified from fish and shellfish (Ibraheem et al., 2017; Elkamouny et al., 2020; Woo et al., 2022). Pathogenic isolates of *P. fluorescens* and *A. hydrophila* exhibit zoonotic potentiality and hemolytic and proteolytic capabilities, and they rapidly develop antibiotic resistance (Thayumanavan et al., 2003; Shayo, 2012).

Many previous studies reported the high resistance of the *Aeromonas* isolates to streptomycin, erythromycin, cefotaxime, amikacin, nalidixic acid, kanamycin, and sulfamethoxazole (Dhanapala et al., 2021; Morshdy et al., 2022). The same finding was reported by Fauzi et al. (2021), who reported the resistance of *Ae*romonas to Kanamycin and agreed with Odeyemi and Ahmad (2017) who extremely observed resistance to penicillin. However, the high sensitivity of the *A. hydrophila* isolates against tetracycline, doxycycline, and ciprofloxacin previously recorded by Zobayer Rahman et al. (2021). Results from this study disagree with the finding of several studies that recorded resistance of the *Aeromonas* isolated to ampicillin (Muila et al. 2021). Discordances in resistance or sensitivity in the literature may be due to differences in isolated sources, frequency, and types of antimicrobial medicines used to treat certain diseases in different geographical areas (Nagar et al., 2011).

The resistance to antibiotics in *A. hydrophila* is both chromosomally and plasmid-mediated; they carry stable plasmid playing a principal role in microbial resistance. The resistance to β-lactam antibiotics may be due to β-lactamases production, which may be coded by plasmids or integrons (Aravena-Román et al., 2012). Resistance to quinolones, such as nalidixic acid, may be caused by chromosomal mutations in the *gyrA* and *parC* genes, which encode target enzymes (DNA gyrase and topoisomerase IV), according to Alcaide et al. (2010).

The *Pseudomonas fluorescens* resistance to erythromycin, penicillin was similar to the result of (Eid et al., 2016). In contrast, sensitivity to gentamicin, ciprofloxacin, tetracycline, ampicillin, doxycycline, amikacin, and cefotaxime was observed in 91.7%, 87.5%, 70.8%, 66.7%, 62.5%, 54.2%, and 50% respectively. Susceptibility to ciprofloxacin, gentamicin, and cefotaxime was reported previously by Darak and Barde (2015) and Eid et al. (2016) and is in line with our results.

The MAR index average of *Aeromonas hydrophila* and *Pseudomonas fluorescens*obtained in this study was in line with that recorded by Morshdy et al. (2022). The antimicrobial resistance trend for bacterial species identified from fish and shrimp sold in the market is startling. Most of the *Aeromonas hydrophila* and *Pseudomonas fluorescens* isolates from aquatic foods were multidrug-resistant.

5. CONCLUSION

In conclusion, we pointed out that the increased resistance to antibiotics of *Aeromonas* and *Pseudomonas* genus inhabiting the fish and shellfish in Egypt was still uncontrolled. The isolated multidrug-resistant *Aeromonas hydrophila* and *Pseudomonas fluorescens* pose a high risk to fish, human, and environmental health. Antibiotic resistance in bacteria might be a major issue in the future, not only in fish health but also in public health because antibiotic resistance might be transmitted to people through fish consumption. Hence, this study indicates that strict regulations and national monitoring programs combined with food safety training for fishermen, vendors, and consumers on various aspects of good hygiene practices are strongly recommended. Further studies are essential for finding a natural alternative for antibiotics to overcome multidrug resistance problems.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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