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Susceptibility to antibiotics and virulence profiling of *Proteus mirabilis* among foodstuff

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Abstract

Proteus mirabilis is a genus of bacteria that can cause foodborne diseases. We collected 100 canned food samples from local supermarkets in Hillah (Iraq), including juice (23%), milk products (22%), beef (20%), fish (14%), milk (12%), and tomato paste (9%) samples. We subsequently characterized some virulence factors of *P. mirabilis* found in these foodstuff, including biofilm formation, protease activity, motility, haemolysis, adhesion, urease production, lipase production, and antibiotic susceptibility. In a total of 32 foodstuff samples of positive culture, *P. mirabilis* was isolated in 15 (46.8%), including 3 juice samples, 4 milk product samples, 2 beef samples, 1 fish sample, 3 milk samples, and 2 tomato paste samples. All isolates exhibited swarming motility (100%) and urease production (100%), while none of the isolates was found to produce haemolysin. The results of the antibiotic susceptibility test revealed a higher resistance against ampicillin (86.6%).

KEYWORDS

Proteus mirabilis, canned foods, milk, virulence factors, antibiotic susceptibility

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1. INTRODUCTION

Food-borne infections are the most prevalent health issue in our country [1]. *Proteus mirabilis* is characterized by several virulence factors that enable it to invade, damage tissues, and evade immunity. The increased wrong use of most antibiotics has been accompanied by the emergence of strains characterized by high resistance, which is increasingly extended to include a wide range of antibiotics [2]. The present study aimed to characterize some virulence factors of *P. mirabilis* isolated from different canned foods collected from the supermarkets in the city of Hillah, Iraq.

2. MATERIALS AND METHODS

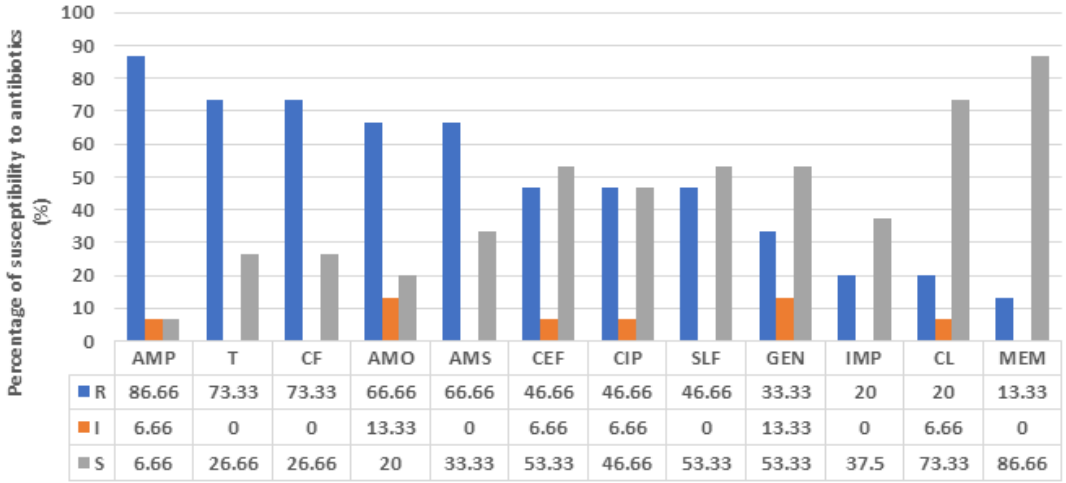
Samples' collection and bacterial isolates: A total of 100 canned food samples from local supermarkets in Hillah (Iraq), including juice (23%), milk products (22%), beef (20%), fish (14%), milk (12%), and to-

mato paste (9%) samples were randomly collected. The isolation and identification of bacterial isolates were carried out by culture and incubation at 37°C for 24 h.

Detection of some virulence factors: *Biofilm formation*: using a tube test, the optical density was measured at a wavelength of 600 nm in test tubes, and the visible line was interpreted as a positive for biofilm potential [2]. *Extracellular protease activity*: skim milk agar containing NaCl was used in order to test the ability of bacteria to produce protease after being incubated at 37°C for 48 h [2]. *Testing of swarming motility*: this test was carried out in Luria-Bertani medium with 0.7% agar, so as to test the swarming motility [3]. *Haemolytic activity*: the presence or absence of haemolysis was determined after the bacteria had been incubated on blood agar for 24 h [3]. *Adhesion of bacteria to epithelial cells*: the result is considered positive when bacteria are attached to the surface of epithelial cells in a single or combined form [4]. *Urease production*: *P. mirabilis* isolates were grown on urea agar medium by stabbing; the tubes were incubated at 37°C for 24–48 h, and the change in the colour from yellow to pink indicated a positive result [4]. *Lipase production*: bacteria cultured on egg yolk agar were inoculated with a bacterial suspension, and were incubated for 24 h at 37°C; the appearance of the decomposition around the colonies was positive [2]. *Antibiotic susceptibility test*: this was carried out by following the Kirby-Bauer method, and the diameter of the inhibition zone was measured [5].

3. RESULTS AND DISCUSSION

Several conventional biochemical tests were performed in order to characterize the suspected *Proteus* isolates, and the results indicated that these isolates were primarily identified as *P. mirabilis*. In a total of 32 foodstuff samples of positive culture, *P. mirabilis* was isolated in 15 (46.8%), including 3 juice samples, 4 milk product samples, 2 beef samples, 1 fish sample, 3 milk samples, and 2 tomato paste samples.



	AMP	T	CF	AMO	AMS	CEF	CIP	SLF	GEN	IMP	CL	MEM
R	86.66	73.33	73.33	66.66	66.66	46.66	46.66	46.66	33.33	20	20	13.33
I	6.66	0	0	13.33	0	6.66	6.66	0	13.33	0	6.66	0
S	6.66	26.66	26.66	20	33.33	53.33	46.66	53.33	53.33	37.5	73.33	86.66

Figure 1. Synopsis of the results of the undertaken antibiotic susceptibility test. Abbreviations used: AMO, amoxicillin / clavulanic acid; AMP, ampicillin; AMS, ampicillin / sulbactam; CEF, cefazolin; CF, cefepime; CIP, ciprofloxacin; CL, chloramphenicol; GEN, gentamicin; I: intermediate susceptibility; IMP, imipenem; MEM, meropenem; R: resistant; S: sensitive; SLF, sulfamethoxazole / trimethoprim; T, tetracycline.

Our results have shown that 9 of the isolates (60%) were able to produce a biofilm by the tube method. Foodborne bacteria develop biofilms in order to survive in a range of unfavourable environments, which are commonly a cause of continuing contamination and outbreaks of foodborne illness. Biofilms enhance the bacterial resistance to environmental stress in the food industry, including the resistance to washing, disinfection, and inhibition, thereby allowing microorganisms to continue to survive on the substrate during the industrial processes [6]. The *P. mirabilis* isolates were unable to produce protease and haemolysin, but they were able to adhere to epithelial cells and generate urease. Moreover, all isolates exhibited swarming motility. The latter is important because

it is coupled with the expression of virulence-associated genes and the ability to invade cells [7]. All 15 of the *P. mirabilis* isolates' strains were able to generate urease; an enzyme that hydrolyses urea so as to produce molecules of ammonia and carbonate, which automatically break down to produce another molecule of ammonia and carbonic acid. This raises the pH and increases the base of the urine, thereby resulting in the formation of stones. This finding was in agreement with those of previous studies [3,7,8]. The results of the undertaken antibiotic susceptibility test are presented in Figure 1. It is believed that the spreading of multi-drug resistance to antibiotics is related to the misuse of antibiotics in humans and animals, especially in countries where it is easy to purchase drugs without the need of pharmacies, as is the case in most developing countries.

4. CONCLUSION

It was found that the *P. mirabilis* isolated from canned food is characterized a number of virulence factors that increase the bacterium's ability to resist the conditions used in canning, in addition to its multi-drug resistance.

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CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

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